

May 1999 (21.05.99)

REC'D 03 JUN 1999

WIPO PCT

*Bureau canadien
des brevets*

*Canadian Patent
Office*

Certification

Certification

09 / 67 4237

La présente atteste que les documents
ci-joints, dont la liste figure ci-dessous,
sont des copies authentiques des docu-
ments déposés au Bureau des brevets.

This is to certify that the documents
attached hereto and identified below are
true copies of the documents on file in
the Patent Office.

Specification and Drawings, as originally filed, with Application for Patent Serial No:
2,230,201, on April 27, 1998, by **HSC RESEARCH AND DEVELOPMENT LIMITED
PARTNERSHIP**, assignee of Sean E. Egan, Wei Wang and Ameet Sengar, for "Ese
Genes and Proteins".

**PRIORITY
DOCUMENT**

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

S. Sengore
Agent certificateur / Certifying Officer

May 21, 1999

Date



Industrie
Canada

Industry
Canada

(CIPO 68)

Canada

ESE GENES AND PROTEINS

Field of the Invention

This invention relates to novel mammalian proteins identified as Ese1 and Ese2
 5 which are involved in endocytosis via clathrin coated pits.

Background of the Invention

Endocytosis via clathrin-coated pits is a multistep process (1, 2). Clathrin heavy
 and light chains are brought to the plasma membrane through association with a
 10 heterotetrameric complex known as clathrin adaptor complex 2, or AP-2. At coated pits,
 the membrane is bent through the assembly of clathrin triskelion into a caged lattice. The
 GTPase Dynamin is also recruited to the neck of coated pits where it assembles into a
 collar for vesicle fission (4, 5). Recruitment of Dynamin to coated pits is believed to
 require the Amphiphysin I/II heterodimer (6), as these proteins bind Dynamin *in vivo* and
 15 amphiphysin proteins are required for endocytosis in yeast (7). In addition, ectopic
 expression of either Amphiphysin I or II by themselves (6), or the isolated SH3 domain of
 Amphiphysin I blocks endocytosis (8, 9). Recent data has revealed that the Rab5 small
 GTPase is required for sequestration of ligands such as transferrin and Epidermal Growth
 Factor into coated pits *in vitro* (10). The mechanism by which these components interact
 20 to regulate coated pit assembly, cargo sequestration, followed by vesicle fission is not yet
 understood.

From biochemical, cell biological and genetic analysis it is clear that additional
 components such as kinases, phosphatases, ubiquitin conjugating enzymes as well as lipid
 modifying enzymes are required for clathrin-coat and vesicle formation (1, 11, 12, 13, 14,
 25 15, 16, 17, 18, 19). Indeed there is also strong evidence for a requirement of the actin
 cytoskeleton in endocytosis and several proteins which may facilitate this connection (1, 7,
 20, 21, 22, 23, 24, 25).

The Eps15 protein was discovered in a search for substrates of the Epidermal
 Growth Factor Receptor(26). In 1995, Benmerah et. al. reported that Eps15 is
 30 constitutively associated with α -adaptin of the AP2 complex (27). The Eps15 protein has
 also been localized to the neck of clathrin-coated pits by immunoelectron microscopy (28,
 29). Recently, two groups have used dominant inhibitory mutants of Eps15, or antibodies
 against Eps15 (or the related protein Eps15R), to demonstrate that Eps15 proteins are
 required for endocytosis via clathrin-coated pits (30, 31). Eps15 contains three large

structural domains(26, 32). The N-terminal third contains three copies of an EH domain (for Eps15 Homology domain) (32, 33). The central region of Eps15 forms an extended coiled-coil, which is followed by a complex C-terminus containing SH3-binding motifs (34), a large number of DPF repeats (Aspartic acid-Proline-Phenylalanine) of unknown function, and α -adaptin binding sequences (31, 35, 36). The full length Eps15R protein has a similar overall organization (34). Both Eps15 and Eps15R can be alternatively spliced to produce numerous smaller proteins(37).

A protein with similar overall organization has been identified in *Saccharomyces cerevisiae* named Pan1p. Genetic analysis of *PAN1* has revealed that this gene is required for endocytosis and for organization of the actin cytoskeleton (23, 38). Like Eps15 and Eps15R in mammals, the Pan1p protein has N-terminal EH domains followed by a central coiled-coil domain and C-terminal proline-rich sequences. A second EH domain containing protein, End3p, has also been described in *S. cerevisiae* which is required for endocytosis and regulation of the actin cytoskeleton (21, 39). Co-immunoprecipitation studies have shown that Pan1p and End3p form a complex *in vivo* (40). Indeed, overexpression of End3p can suppress the phenotype of *pan1-4* hypomorphic mutants, and Pan1p is mislocalized in *end3* mutants indicating that these proteins function together (40). Additional studies have revealed that the EH domains of Pan1p bind to yeast homologues of mammalian clathrin-binding proteins, AP180 and CALM (yAP180A and yAP180B), through NPF motifs (Asparagine-Proline-Phenylalanine) in the yAP180 C-termini(25). These data have led to a proposal that the Pan1p:End3p complex functions as a multivalent adaptor to coordinate protein-protein interactions during endocytosis (25, 40). At least two additional proteins are predicted to bind to the Pan1p:End3p complex *in vivo*, as strong genetic interactions have been detected between *PAN1* and *SJL1*(25), and between *PAN1* and *RSP5* (41). *SJL1* encodes a phosphatidylinositol polyphosphate-5-phosphatase protein which is related to mammalian synaptojanin (42) and has a C-terminal NPF motif predicted to bind to EH domains in Pan1p (or End3p) (25, 43). *RSP5* encodes an E3 ubiquitin-protein ligase which may bind to the C-terminal polyproline sequences in Pan1p through one of its three WW domains (25).

Numerous SH3 domain containing proteins have been implicated in the regulation of endocytosis (44). These include Amphiphysin I(45) and II (6, 46, 47, 48), Rsv161/Rsv167(7), Actin Binding Protein-1(49), Endophilin/SH3P4/8/13 (50, 51) and Grb2 (52). Kay and coworkers have reported the isolation of several novel SH3 encoding cDNAs (53).

The present inventors have identified novel mammalian proteins containing both EH and SH3 domains, which have been named Ese1 and Ese2. Sequence and functional analysis of the full length proteins have implicated these proteins in both pinocytosis and receptor mediated endocytosis via clathrin coated pits and therefore the proteins have been
5 named Ese1 and Ese2 respectively (Ese: for EH-domain and SH3 domain regulator of Endocytosis).

Summary of the Invention

In accordance with one series of embodiments, this invention provides isolated
10 nucleic acids corresponding to or relating to the nucleic acid sequences disclosed herein which encode the murine Ese1 and Ese2 proteins.

One of ordinary skill in the art is now able to identify and isolate mammalian Ese protein genes or cDNAs which are allelic variants of the disclosed sequences or are homologues thereof, in other species, including humans, using standard hybridisation
15 screening or PCR techniques.

In a further embodiment, the invention provide cDNA sequences encoding murine Ese1 and Ese2 proteins comprising the nucleotide sequences of Sequence ID NOS: 1, 2, 4 and 5.

Also provided are portions of the Ese gene sequences useful as probes or PCR
20 primers or for encoding fragments, functional domains or antigenic determinants of Ese proteins.

The invention also provides portions of the disclosed nucleic acid sequences comprising about 10 consecutive nucleotides to nearly the complete disclosed nucleic acid sequences. The invention provides isolated nucleic acid sequences comprising sequences
25 corresponding to at least 10, preferably 15 and more preferably at least 20 consecutive nucleotides of the Ese genes as disclosed or enabled herein or their complements.

In addition, the isolated nucleic acids of the invention include any of the above described nucleotide sequences included in a vector.

In accordance with a further series of embodiments, this invention provides
30 substantially pure murine Ese proteins, fragments of these proteins and fusion proteins including these proteins and fragments.

In accordance with a further series of embodiments, this invention provides substantially pure mutant murine Ese proteins, fragments of these proteins and fusion proteins including these mutant proteins and fragments.

The proteins, fragments and fusion proteins have utility, as described herein, for the preparation of polyclonal and monoclonal antibodies to mammalian Ese proteins, for the identification of binding partners of the mammalian Ese proteins and for diagnostic and therapeutic methods, as described herein. For these uses, the present invention provides

5 substantially pure proteins, polypeptides or derivatives of polypeptides which comprise portions of the mammalian Ese amino acid sequences disclosed or enabled herein and which may vary from about 4 to 5 amino acids to the complete amino acid sequence of the proteins. The invention provides substantially pure proteins or polypeptides comprising

10 sequences corresponding to at least 5, preferably at least 10 and more preferably 50 or 100 consecutive amino acids of the mammalian Ese proteins disclosed or enabled herein.

The proteins of the invention may be isolated and purified by a conventional method suitable in relation to the properties revealed by the amino acid sequences of these proteins.

Alternatively, cell lines may be produced which express or over-express the Ese

15 gene products, allowing purification of the proteins for biochemical characterisation, large-scale production, antibody production and patient therapy.

For protein expression, eukaryotic or prokaryotic expression systems may be generated in which an Ese gene sequence is introduced into a plasmid or other vector which is then introduced into living cells. Constructs in which the Ese cDNA sequence

20 containing the entire open reading frame is inserted in the correct orientation into an expression plasmid may be used for protein expression. Alternatively, portions of the sequence may be inserted. Prokaryotic or eukaryotic expression systems allow various important functional domains of the proteins to be recovered as fusion proteins and used for binding, structural and functional studies and also for the generation of appropriate

25 antibodies.

The present invention includes effective fragments, analogues of the Ese proteins described herein. "Effective" fragments or analogues retain the activity of the described Ese proteins to promote endocytosis via clathrin-coated pits. The term "analogue" extends to any functional and/or chemical equivalent of a mammalian Ese protein including

30 mimetics and includes proteins having one or more conservative amino acid substitutions, proteins incorporation unnatural amino acids and proteins having modified side chains.

In accordance with a further embodiment of the invention, antibodies are enabled which bind specifically to the Ese proteins disclosed herein. Polyclonal or monoclonal

antibodies may be prepared using conventional methods. Antibodies may also be prepared to individual selected domains of the Ese proteins, as described herein.

5 In a further embodiment, the invention provides pharmaceutical compositions containing an Ese protein or a non-functional mutant Ese protein for the treatment of mammalian disorders which involve abnormal endocytosis leading to altered cellular functioning. Administration of a therapeutically active amount of a pharmaceutical composition of the present invention means an amount effective, at dosages and for periods of time necessary to achieve the desired result.

10 In accordance with a further embodiment, the invention provides a method for identifying binding partners of the Ese proteins disclosed herein. Such methods in general include various assays including those including radiolabelling of the Ese proteins. Other methods may include but are not restricted to phage display, affinity purification techniques, expression cloning and the yeast 2-hybrid system, as described herein.

15 In accordance with a further embodiment of the present invention, is a method for identifying proteins which phosphorylate Ese proteins. Such method includes known phosphorylation assays.

20 The identification of proteins or peptides interact with or bind to Ese proteins can provide the basis for the design of peptide antagonists or agonists of Ese protein function or for the design of peptide antagonists or agonists of Ese protein binding partners which affect Ese protein function. Further, the structure of these peptides determined by standard techniques such as protein NMR or x-ray crystallography can provide the structural basis for the design of improved small molecule drugs.

25 In accordance with a further embodiment, the present invention also provides for the production of mouse models or transgenic non-human animal models for the study of mammalian Ese gene function, for the screening of candidate pharmaceutical compounds, for the creation of *in vitro* mammalian cell cultures which express the Ese proteins or in which an Ese gene has been inactivated by knock-out deletion, and for the evaluation of potential therapeutic interventions.

30 The invention enables a transgenic animal, including a transgenic insect, wherein the genome of the animal or of an ancestor of the animal has been modified by introduction of a transgene comprising mammalian Ese genes under the transcriptional control of tissue restricted regulatory elements including the mouse mammary-tumour virus long term repeat sequences.

Transgenic animals with inappropriate expression of Ese proteins may be examined for phenotypic changes, for example abnormal cellular development or abnormal cellular signalling and may be used to screen for compounds with potential as pharmaceuticals. Compounds which provide reversal of the phenotypic changes are candidates for development as pharmaceuticals.

Transgenic animals in accordance with the invention can be created by introducing a DNA sequence encoding a selected Ese protein either into embryonic stem (ES) cells of a suitable animal such as a mouse, by transfection or microinjection, or into a germ line or stem cell by a standard technique of oocyte microinjection. Such methods of producing animal models are fully described in the literature.

In accordance with another aspect of the present invention is a method for screening a candidate compound for effectiveness as an antagonist of an Ese protein comprising:

- (a) providing an assay method for determining the endocytotic regulatory capacity of an Ese protein; and
- (b) determining the endocytotic regulatory capacity of the Ese protein in the presence or absence of the candidate compound, wherein a reduced level of endocytosis in the presence of the candidate compound indicates antagonist activity of the compound.

In accordance with another aspect of the present invention is a method for treating in a mammal a disorder associated with an undesired level of endocytotic activity of an Ese protein comprising administering to the mammal an effective amount of a substance selected from the group consisting of:

- (a) an Ese protein antagonist;
- (b) an antibody which binds specifically to an Ese protein;
- (c) an antisense strand comprising a nucleic acid sequence complementary to the sequence or fragment of the sequence and capable of hybridizing to the nucleic acid sequence encoding an Ese protein;
- (d) an agent which down regulates the expression of the Ese gene encoding for an Ese protein;
- (e) an antagonist of an Ese protein binding partner; and
- (f) an Ese agonist.

According to another aspect of the present invention is a method for suppressing in a mammal, the abnormal proliferation of a cell capable of being stimulated to proliferate by

a growth factor receptor, the method comprising administering to the mammal an effective amount of a Ese protein antagonist, an Ese agonist or an antibody which binds specifically to an Ese protein.

According to yet another aspect of the present invention is a method for preventing viral infection in a mammal, said method comprising administering to the mammal an effective amount of an Ese protein antagonist, an Ese agonist or an antibody which binds specifically to an Ese protein or an Ese mutant protein not capable of regulating endocytosis.

According to a further aspect of the present invention is a method for causing endocytosis in selected cells in a mammal in need of such treatment, said method comprising administering to the mammal an effective amount of an Ese protein or an active analogue, mimic or fragment thereof.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

20 **Summary of Tables and Drawings**

A detailed description of the preferred embodiments are provided herein below with reference to the following tables and drawings in which:

Table 1 shows alignment of mouse Ese1 and Ese2 protein sequences. EH, coiled-coil and SH3 domains are as indicated.

25 Figure 1 shows a Northern blot demonstrating expression of Ese1 and Ese2 genes in various adult tissues.

Figure 2 shows Western blots demonstrating the association of Ese and Eps15 proteins *in vivo*. Endogenous Ese1 proteins were precipitated with Rabbit anti-peptide antisera against the N-terminus of Ese1. Immunoprecipitates were then analyzed on western blots for the presence of Ese1/2 with Chicken anti-Ese antisera or for co-precipitation of Eps15 with Rabbit antisera raised against the C-terminus of Eps15.

Figure 3 shows association of Ese and Eps15 C-terminal deletion mutants. Cos-1 cells were transfected with pcDNA3Eps15, pcDNA3Ese1 or the C-terminal deletion mutants pcDNA3Ese1 δ C and pcDNA3Eps15 δ C-Flag as indicated. Cell lysates were

immunoprecipitated with rabbit anti-Ese1 or rabbit anti-Eps15 (left panel); rabbit anti-Ese1, mouse anti-Flag or rabbit anti-Eps15 (right panel). Panels represent western blots to detect the presence of Ese1 in each immunoprecipitation. A 90kDa Ese1 protein exists in the third and sixth lanes on the left panel which is the C-terminally truncated Ese1 protein which is co-immunoprecipitated in a complex with Eps15 in the sixth lane. Also, to be noted is the precipitation of Ese1 with anti-Flag monoclonal antibody in the sixth lane of the right panel experiment. In this case, Ese1 has been precipitated in a complex with the C-terminally truncated Eps15 δ C protein.

Figure 4 shows confocal immunofluorescent micrographs of Cos cells transfected with myc-Ese1 (A, A'); Eps15 (B); myc-Ese1 + Eps15 (C, C', C'', D) or myc-Ese1 + Eps15 δ C (E).

Figure 5 shows confocal immunofluorescent micrographs of Cos cells transfected with myc-Ese1 and probed with antibodies to myc (A), Dynamin (B) or both antibodies (C).

Figure 6 shows a proposed model for Ese regulation of Endocytosis.

In the drawings, preferred embodiments of the invention are illustrated by way of example. It is to be expressly understood that the description and drawings are only for the purpose of illustration and as an aid to understanding, and are not intended as a definition of the limits of the invention.

Detailed Description of the Invention

Sequencing of Murine Ese1 and Ese2 genes

The full length murine Ese1 cDNA was sequenced (Sequence ID NO:1). It encodes a sequence of 1213 amino acids (Sequence ID NO:3) having a predicted molecular weight of 137 kDa. Murine Ese2 cDNA was also sequenced (Sequence ID NO:4) and encodes a sequence of 1197 amino acids (Sequence ID NO:6) having a predicted molecular weight of 135.7 kDa.

The full length Ese proteins are predicted to encode two N-terminal EH domains followed by a coiled-coil domain and five SH3 domains (Table 1). Several Ese1 isolated clones contain only SH3_A, SH3_B and SH3_E domains. Indeed, the SH3_C domain was not included in the original human SH3P17 partial cDNA. In addition, expressed sequence tags from the Ese1 gene have been found in the public databases which skip sequences encoding individual EH domains or regions of the coiled-coil domain indicating that this gene is subject to complex alternative splicing and has the potential to encode for many

distinct proteins. The predicted Ese2 protein on the other hand encodes a C-terminal extension of 45 amino acids in comparison to the human SH3P18 partial cDNA, suggesting that this gene is also alternatively spliced. The Ese proteins are 53% identical over the full length of Ese1 (645 of 1213 residues in Ese1 line up with identical residues in Ese2) and are related to the Ese protein from *Xenopus* which has recently been submitted to genebank (Accession # AF032118).

The two EH domains of Ese1 are highly related to the respective EH domains in Ese2 and these Ese EH domains are most closely related those found in Eps15 and Eps15R, two mammalian protein which are required for endocytosis through clathrin-coated pits. EH domains have also been identified in End3 and Pan1p which are yeast partners proteins required for endocytosis. The central third of both Ese proteins are predicted to encode an extended coiled-coil which is a domain typically associated with protein-protein association through dimerization or tetramerization as noted for Eps15 (29, 54). Besides the homology between Ese1 and Ese2, the Ese1 SH3 domains are most closely related to SH3 domains from Myosin IB in *Acanthamoeba* (SH3_A), Myosin IB in *Entamoeba* (SH3_B), the YFR024 hypothetic yeast protein (SH3_C), Myosin IB from *Acanthamoeba* (SH3_D) and Myosin IC from *Acanthamoeba* (SH3_E). The same homologies are noted for SH3 domains from Ese2 with the exception of SH3_A which is most similar to the SH3 domain from β PIX, SH3_C which is most similar to an SH3 domain from the mouse Ray protein and SH3_D which is most similar to the SH3 domain from Dictyostelium myosin IB. Additional sequence analysis reveals the presence of a very large number of potential phosphorylation sites in the Ese proteins and a single SH3 binding consensus in the N-termini of each Ese protein.

25 Expression of Ese genes

In order to determine where the Ese genes are expressed we have performed Northern analysis on mRNA derived from several adult mouse tissues (Figure 1). These genes are both widely expressed and reveal a complex pattern of alternatively spliced transcripts. The highest levels of Ese1 mRNA were noted in brain, heart and skeletal muscle. Brain and skeletal muscle mRNA also contain an unusually large transcript which may be as much as 15kb in length. Interestingly, these tissues express high levels of many proteins involved in synaptic vesicle recycling and endocytosis. A prominent 2.4kb Ese2

transcript has also been noted which is significantly smaller than the 3591bp sequence required to code for the full Ese2 protein as shown in Table 1.

Subcellular localisation of Ese proteins

To determine the subcellular localization of Ese1, a myc-epitope tagged version of this protein (mycEse1) was engineered. This tagged protein was expressed in Cos cells and detected by confocal immunofluorescence using the mouse anti-myc monoclonal antibody 9E10 (Figure 4). Interestingly, the transfected Ese1 protein is highly concentrated into circular domains (Figure 4A and 4C) which are present throughout transfected cells

(compare single optical section in Figure 4A with the projection of all sections through the same cell shown in 4A'). Ese1 overexpressed in 10T1/2, BHK and Hela cells using the vaccinia virus T7 expression system is also localized to large circular domains (data not shown). This concentration of ectopically expressed Ese1 contrasts with the localization of Eps15 in transiently transfected cells, Eps15 being dispersed throughout the cell (Figure 4B). Ese1 and Eps15 proteins form a complex *in vivo* and yet localize to distinct subcellular compartments in transfected Cos cells. We therefore determined the localization of both mycEse1 and Eps15 in Cos cells co-transfected with both genes. In co-transfected cells, mycEse1 is still found in circular domains (Figure 4C). Interestingly, the transfected Eps15 is now also partially co-localized with Ese1 in the same circular domains (Figure 4C' and C'').

The C-terminal third of Eps15 contains several regions which are required for association with α -adaptin of the AP2 clathrin adaptor complex (31). It has been shown that this region of Eps15 is not required for its association with Ese1 (Figure 4). In order to test whether Eps15 function may be required for Ese localization, mycEse1 and Eps15 δ C have been co-transfected. Interestingly, mycEse1 localization is completely altered in cells co-transfected with Eps15 δ C (compare Figure 4D and E) indicating that Ese1 requires Eps15 function for its distinctive subcellular localization. In addition, these data suggest that the Ese1:Eps15 complex must be associated with AP2 in order to form the large circular domains in Ese1 transfected cells.

The Dynamin GTPase is required for clathrin-coated vesicle fission and is recruited to coated pits by Amphiphysin I/II heterodimers (6). In order to study the subcellular localization of Dynamin in Ese1 overexpressing cells we have co-stained for the myc epitope tag with Rabbit anti-myc antisera and for endogenous Dynamin with mouse anti-Dynamin monoclonal antibodies. Surprisingly, endogenous Dynamin is recruited to the Ese1 staining circles and both proteins are co-localized (Figure 5). These results

demonstrate that mycEse1-containing circles are endocytic structures and that Ese1 is a rate limiting component of the early endocytic compartment which can function together with Eps15 to induce Dynamin recruitment.

5 Identification of binding partners of Ese proteins

In order to identify Ese partners, a systematic analysis was initiated of each domain for binding partners using the yeast two hybrid system. The central coiled-coil domain of Ese1 from amino acid 330 to 732 was fused to the GAL4 DNA binding domain and transformed into the Y190 reporter strain together with plasmid cDNA libraries from
 10 several tissue sources. Yeast colonies were selected for growth on Histidine minus plates to select for interaction between library encoded GAL4 activation domain fusions and the GAL4 DNA binding domain Ese1 coiled-coil bait. Yeast colonies which survived selection for expression of the His3 gene were also tested for induction of the integrated LacZ gene which is GAL4 responsive in Y190. Prey plasmids were recovered from 37
 15 positive yeast colonies which were selected for expression of both His3 and LacZ. Several classes of cDNA were recovered in this screen. One class included Eps15 which was isolated twice and Eps15R which was isolated from four yeast colonies. Interestingly these clones were all partial cDNA fusions which minimally included C-terminal sequences from the central coiled-coil domain and downstream. In the case of Eps15, the positive
 20 clones start from amino acid 306 and 376 whereas in Eps15R the N-terminal boundary of clones were amino acid 4, 10, 222 and 386. These data indicate that minimal sequences required for interaction between the Ese1 coiled-coil domain and Eps15(R) include either or both of the central coiled-coil and/or the C-terminal third of these proteins.

The Ese proteins were analyzed *in vivo* to test for their association with Eps15 or
 25 Eps15R. Polyclonal antisera were raised in chickens against a GST fusion containing the C-terminus of Ese1 from amino acid 665 to the stop codon. This region of Ese1 contains all five of the SH3 domains. In addition, polyclonal antisera were generated in rabbits against a peptide representing the first 21 amino acids of Ese1. Cell lysates were prepared from A431, PC12, MDCK and Hela cells which represent cell lines from several distinct
 30 tissue types and species. The rabbit anti-peptide antisera were used to precipitate Ese1 from each lysate and precipitates were analyzed by western blotting using the chicken anti-Ese sera. In each cell line the presence of several specific bands in the range of 150kDa were observed which were precipitated in the absence but not in the presence of the peptide to which the sera was generated (Figure 2). Thus the Ese1 protein is expressed in

many tissue culture cells and runs in a range consistent with the predicted molecular weight of 137kDa. The same samples were also analyzed for co-immunoprecipitation of Eps15 proteins. In each case multiple Eps15 proteins were detected which co-purify with Ese1. The anti-Ese1 peptide antisera and commercially available anti-Eps15 antisera
 5 which were used were raised against epitopes which are not shared by these proteins indicating that Ese1 and Eps15 are constitutively associated partners *in vivo*. This association is reminiscent of the previously detected complex between two EH domain containing yeast proteins, End3p and Pan1p.

Ese1 and Eps15 proteins both contain central coiled-coil motifs. In addition, Ese1
 10 contains multiple C-terminal SH3 domains while Eps15 contains SH3-binding motifs. In order to map the regions of each protein which are required for their association *in vivo*, C-terminal truncations of each (Eps15 δ C and Ese1 δ C) were generated. Full length Eps15 was co-transfected into Cos-1 cells together with either full length Ese1 or C-terminally truncated Ese1 δ C. Cell lysates were precipitated with either rabbit anti-Eps15 or with
 15 rabbit anti-Ese1, and precipitates were western blotted with chicken anti Ese1 antisera. Interestingly, the C-terminally truncated Ese1 protein was efficiently immunoprecipitated in a complex with Eps15 using anti-Eps15 sera. In a reciprocal experiment Cos-1 cells were transfected with Ese1 alone or together with Eps15 or C-terminally truncated Eps15 which had been Flag-epitope tagged (Eps15 δ C-Flag). Cell lysates were prepared and
 20 immunoprecipitated with either rabbit anti-Ese1, mouse anti-Flag, or rabbit anti-Eps15 antibodies. These immunoprecipitations were also western blotted to analyze for the presence of Ese1. The anti-Flag antibody efficiently precipitated Ese1 from cells expressing Flag-tagged Eps15 δ C indicating that the C-terminally truncated Eps15 protein can bind to Ese1 *in vivo*. The Ese1 and Eps15 proteins are therefore associated through
 25 interaction of their central coiled-coil regions and do not require the presence of SH3 and SH3-binding motifs in their respective C-termini.

In addition to the identification of Eps15 and Eps15R in the yeast two hybrid screen with the coiled-coil domain bait of Ese1, the following were also identified as Ese binding proteins: TSG101 (accession #U52945), meningioma expressed antigens 6/11
 30 (accession #U94780 for mea6), β -tropomyosin, rabaptin5 (accession #D86066), Adora2a (accession #Y13345), L1 lipid binding protein (accession #K02109) and numerous cytokeratins and laminins. Novel genes identified in this screen are detailed below.

Also performed was a yeast two hybrid screen using amino acids 665-1213 of Ese1 as bait. This screen led to the isolation of the following clones which produced GAL4 Activation domain fusions which bound to this SH3 domain bait of Ese1. The Ese-binding proteins identified in our SH3 screen were the cbl-b oncoprotein (accession #U26712),
 5 Dynamin II (accession #L31398), KIAA0268 (accession #D87742), Jerky (accession #U35730), hnRNP-K (accession #L29769), SAP49 (accession #L35013). Novel genes identified in this screen are detailed below and several novel clones as outlined below.

These results demonstrate that the novel Ese genes and the proteins that they
 encode function in a complex with Eps15 proteins to regulate endocytosis together. In
 10 addition, this complex contains binding sites for numerous other proteins. Furthermore, with the identification of several potential phosphorylation sites on the Ese proteins, these results also suggest that whether or not complexed with Eps15, Ese proteins are involved in intracellular signalling processes which are likely to lead to altered cellular activity. Many Ese partners have been identified in these studies. Also revealed is a novel method
 15 to identify more Ese partners. Yeast cells have a complex formed by two EH domain proteins (Pan1p and End3p) which regulates both endocytosis as well as the actin cytoskeleton. Indeed, the Eps15 protein has been reported to regulate both endocytosis and the actin cytoskeleton (1). As Eps15 and Ese function together, and Ese contain many protein-protein interaction surfaces on this complex, strongly suggesting that the Ese
 20 proteins and their binding proteins are critical regulators of Eps15:Ese functions *in vivo*.

Taken together, these studies demonstrate the identification and isolation of two novel murine Ese proteins encoded by two novel murine Ese genes which are involved in the regulation of endocytosis via clathrin-coated pits. The process of endocytosis is most likely to be related to receptor-mediated endocytosis, however, may also be related to
 25 pinocytosis or non-receptor mediated endocytosis. The novel Ese proteins of the present invention appear to function to regulate endocytosis involving the formation of clathrin-coated pits by the polymerization of clathrin into a lattice along the cytosolic face of a region of membrane causing the region to expand inward. Ultimately, the pit pinches off from the membrane, and the clathrin cage is completed thus producing a coated vesicle.
 30 Through the binding of Eps15 to form an Ese-Eps15 complex via a central binding region, the complex can recruit other proteins such as assembly particles and dynamin to bind GTP and promote coated vesicle formation and perhaps their transport to appropriate locations within the cell and release of internalized proteins and/or molecules. The SH3 C-terminal domains of the Ese proteins can bind and interact with several other proteins

leading to a host of protein-protein interactions involved in the endocytotic process. The Ese proteins may be activated or inactivated via phosphorylation of the proteins at numerous phosphorylation sites by the action of activated receptors on cell surfaces.

Due to the fact that the Ese proteins appear to be a key central player in the complex process of endocytosis involving protein-protein interactions and intracellular signalling, these proteins are most likely involved in a myriad of clinical conditions and processes which are very likely to include but not be limited to regulation of endocytosis (as described above), cell division and cancer (Eps15 and cbl-b are oncoproteins), cell migration (regulation of the actin cytoskeleton is required for many forms of cell migration), cell polarity, plane of cell division and cell fate specification (Eps15 binds to Numb *in vivo* which is required for these processes(43), RNA localization (several RNA binding proteins have been identified in the present screens) and viral infection and life cycle (Eps15 binds to RAB a cellular cofactor for HIV Rev (43)).

With respect to viral infection Ese proteins may play an important role and thus may be a target for developing therapeutic strategies against viral infection and virally-induced disease states. HIV is known to alter endocytosis of several important cell surface molecules including CD4 and MHC antigens. HIV has been shown to bind to NEF which has been demonstrated to bind to SH3 sites. This binding to NEF induces clathrin coated pit formation. As NEF binds SH3 sites and induces endocytosis, it is possible that Ese proteins may bind to NEF and are involved in the endocytotic process. Therapeutic strategies to provide treatment for viral infection and virally induced disease states may therefore include the inhibition of Ese-NEF binding using antagonists, antibodies or other agents directed against such a complex to inhibit endocytosis and in this manner inhibit viral infection and virally induced disease states. It is also expected that many types of viruses will interact with the multi-component Eps15-Ese complex.

Synaptic transmission and abnormal or altered synaptic transmission as seen in various nervous disorders may also be a target for the therapeutic use of Ese proteins and/or antagonists. The Eps15:Ese complex has been demonstrated to regulate endocytosis, is highly expressed in the brain and Ese binds to Jerky; a protein required to prevent epilepsy in mice (57). Furthermore, Ese proteins are highly expressed not only in the brain but also in the heart and in skeletal muscle which are tissues involving high levels of synaptic transmission. This suggests that Ese protein may be used in the treatment of nervous system disorders involving altered synaptic transmission.

Receptor-mediated cell signaling such as seen with several different types of growth factors also involves endocytosis. Over-expression, mutation or over-stimulation of growth factor receptors has been demonstrated to lead to abnormal cell division and growth as seen in cancer. For example, EGF is a potent mitogen for many epithelial cells and EGF receptor activation is known to stimulate intracellular kinase pathways leading to cell proliferation. Such activity may play a role in cancer progression. By altering the rate of endocytosis by targeting Ese proteins, the cell proliferative effect of growth factor receptor stimulation may be counteracted.

Abnormal cell division and cell migration is seen in several diseases and involves the cell cytoskeleton. The intracellular cytoskeleton is highly organized and consists of microtubules, microfilaments and intermediate filaments acting as an internal reinforcement in the cytoplasm of a cell. Together these structures associate in a regular and defined manner which is regulated by extracellular signals and may transduce plasma membrane signals by association with other proteins or by second messengers. The Ese-Eps15 complex is very likely to regulate the cytoskeleton by analogy to the role of Pan1p:End3p in regulation of yeast cytoskeleton. Furthermore, endocytosis is known to involve a rearrangement of the intracellular cytoskeleton. Cell division and migration require the continual rearrangement of the intracellular cytoskeleton. Therefore, abnormal patterns of cell division and migration may involve altered Ese function and altered endocytosis. The Ese protein or the gene may therefore be used to alter regulation of endocytosis or the association of the Ese protein with the cell cytoskeleton to restore cell division and migration to normal levels and patterns.

Tissue development also involves the continual remodeling of the extracellular and intracellular cytoskeletal network along with associated proteins. Developmental diseases can occur as a result of abnormal remodeling of the cytoskeleton leading to altered intracellular signaling. As Ese proteins are likely to be involved with both the cytoskeleton and intracellular signaling they may also be directly involved in the development of certain developmental diseases and therefore may be a target for therapeutic treatment of such diseases. Ese proteins may also be involved in normal development including that of stem cells which are self-renewing cells that divide to produce differentiated daughter cells in various tissues. As Ese proteins are associated with the cytoskeleton they may play a part in the formation of certain types of differentiated cells through the partitioning of RNA and proteins such as NuMb during cell division.

To summarize, Ese proteins, Ese complexes including Eps15, Eps15R and many of the proteins identified in the screens as well as others identified through similar screens can be targeted for use in therapies to treat diseases including cancer, viral infection based diseases, developmental diseases due to altered cell fate specification and/or division as well as neurological diseases and diseases of altered cell migration and other diseases due to defects in the actin cytoskeleton.

EXAMPLES

The examples are described for the purposes of illustration and are not intended to limit the scope of the invention.

Methods of molecular genetics, protein and peptide biochemistry and immunology referred to but not explicitly described in this disclosure and examples are reported in the scientific literature and are well known to those skilled in the art.

Material and Methods

Ese cloning and plasmids

High Stringency screening was used to isolate the two mouse Ese cDNAs by previously described methodology (58). Ese1 was cloned from an adult mouse brain cDNA library using a PCR product from nt 1707-2197 of the coding sequence as probe. Ese2 was cloned from a mixed tissue adult mouse cDNA library using a mixture of three probes (EST#583881 (Research Genetics Inc.), EST#652549 (Research Genetics Inc.) and a PCR product from nt 2712 to nt 3456 of the Ese2 coding sequence. The mouse Eps15 cDNA was generated through a combination of high stringency library screening with Est sequences from the Eps15 gene and rt PCR according to established methods.

pcDNA3Ese1:

Full length Ese1 was cloned into the NotI site of pcDNA3 (Invitrogen Inc.). The Ese1 cDNA includes 53 nucleotides of 5' UTR plus a natural NotI site and 288 nucleotides of 3'UTR plus a small region of polylinker including a NotI site.

pcDNA3mycEse1:

The 5' end of pcDNA3Ese1 from the EcoRI site in the pcDNA3 polylinker to the start codon was replaced with the DNA sequence
GAATTCAGAACCATGGAACAAAAGCTTATTTCTGAAGAAGACTTGGGGCCCATG:

where the first underline corresponds to an EcoRI site which was fused into the pcDNA3 EcoRI site and the extended underlined sequence codes for a myc-epitope tag. This is followed by nine nucleotides which code for glycine, proline and the natural Ese1 start codon. This sequence was joined to the sequences coding for amino acids 2-1213 (the remainder of Ese1). The new start codon in this tagged Ese1 construct is bolded. The 3' end of Ese1 in this vector is the same as in pcDNA3Ese1 above.

pcDNA3Ese1δC:

The C-terminus of Ese1 was removed by replacing all sequences from nt 2209 of the coding sequence to the XhoI site in pcDNA3Ese1 with **TGACTCGAG** where the stop codon is in bold and the XhoI site is underlined. This construct codes for amino acids 1-736 of Ese1.

pCDNA3Eps15:

This plasmid was constructed from four pieces. It contains the full length Eps15. The 5'UTR of this construct has been constructed to be GGATCCACCATG where a BamHI site is underlined and the start codon is bolded. This BamHI site was fused to the BamHI site in pcDNA3. The 3'UTR in this vector is 204 nt of the mouse natural 3'UTR fused to a short cloning linker ending in the sequence AAGCTTGGGCCC where an ApaI site is underlined; this ApaI site was fused to the ApaI site in pcDNA3.

pcDNA3Eps15δC:

This vector is the same as pcDNA3Eps15 except that sequences downstream from and including mouse Eps15 coding nucleotide 1500 have been replaced with CCTGGATTACAAGGATGATGATGACAAATGACTCGAG where the first underlined sequence codes for the Flag-epitope, an inframe stop codon is bolded and an XhoI site is underlined. This XhoI site was fused to the polylinker in pcDNA3. The resulting plasmid encodes amino acids 1-501 of mouse Eps15 fused to a C-terminal Flag epitope. The 5' end of Eps15 in this construct is as indicated above for pcDNA3Eps15.

pGBT9Ese1cc:

The Ese1 sequence coding for amino acids 330 to 732 were fused directly to GAATTC (EcoRI site) on the 5' end and to TAGGATCC (stop codon followed by a

BamHI site) on the 3' end. This fragment was cloned into EcoRI/BamHI digested pGBT9 in frame with the GAL4 DBD.

pGBT9Ese1A3.3:

- 5 This plasmid encodes the bait for our SH3 screen. It encodes all five SH3 domains from amino acid 665-1213 and was subcloned into pGBT9 on an EcoRI fragment which fuses the Ese1 SH3 region in frame with the DBD of GAL4.

Yeast 2-Hybrid Screening

- 10 pGBT9Ese1cc was transformed into *S. cerevisiae* strain Y190 using 45% PEG4000, 100 mM LiAc, 10 mM Tris-HCl (pH 7.5) as per standard protocols. Cells were plated onto Sc-Trp drop-out media. Single colonies were isolated and expression of bait was analyzed by Western Blot using antibodies against the GAL4 DBD. A clone expressing the bait fusion was used to inoculate a 100 mL Sc-Trp liquid culture grown
- 15 overnight at 30°C. Cells were then re-inoculated into YPD at a density of 5×10^6 cells/mL and grown at 30°C until the titer reached 2×10^7 cells/mL. Cells were pelleted, resuspended in 50 mL 100 mM LiAc and incubated for 10 min at 30°C. Once again the cells were pelleted, resuspended in 20 mL PLA [35% PEG, 100 mM LiAc, 2 mg/mL salmon sperm
- 20 DNA] containing 30 µg plasmid library (cloned in pAD-GAL4) incubated at 30°C for 30 min.; then heat shocked at 42°C for 40 min., pelleted, resuspended in water and plated onto [Sc-Trp-Leu-His+40 mM 3-AT]. Plates were incubated at 30°C until colonies were formed. Colonies were picked, patched and grown at 30°C overnight on Whatman filter paper laid on top of Sc-Trp-Leu-His plates. Filters were submerged in liquid nitrogen for 15 seconds then placed on top of blotting paper soaked in Z-buffer + X-gal. β-
- 25 galactosidase activity was measured by the appearance of blue colour. Plasmids from β-galactosidase positive colonies were shuttled to bacteria by electroporation and isolated for sequencing.

Northern Blot analysis

- 30 A multiple tissue northern blot (Clontech) was prehybridized in 5 mL of ExpressHyb Solution (Clontech) at 68°C for 30 min. Probe was added at 1×10^6 cpm/mL for 1 hr. The Blot was washed twice [2X SSC, 0.05% SDS] at room temperature, twice [0.1X SSC, 0.1% SDS] at 50°C and then exposed to film overnight.

Antibodies and Western Blot analysis

Western blot analysis was performed according to standard protocols. Briefly, cultured cell lines or 48 hours post transfection Cos-1 cells were washed with PBS and lysed in one ml of cold lysis buffer (50 mM Hepes pH 7.4, 150 mM NaCl, 10% glycerol, 1% Triton X100, 1 mM EGTA, 1.5 mM MgCl₂, 10 mM NaF, 10 mg ml⁻¹ aprotinin, 1 mM PMSF, 10 mg ml⁻¹ leupeptin, 1 mM Na₃VO₄). Supernatants were clarified by centrifugation and immunoprecipitated with specific antiserum as indicated. Antigen-Antibody complexes were purified on anti-rabbit agarose or anti-mouse agarose (Sigma Chemical Co.) Samples were run on 7.5% PAGE gels and transferred to nitrocellulose membranes. Filters were blocked in 5% dry milk powder/0.05% Tween 20/PBS, washed in 1% dry milk powder/0.05% Tween 20/PBS, and probed with the appropriate antisera at 1 mg/ml in wash buffer (In the case of chicken anti-Ese1 we used 10 µg/ml to probe western blots). Probed filters were further washed, probed again with 1/5000 dilution of horseradish peroxidase conjugated anti-rabbit IgG, anti-mouse IgG antibodies (Amersham), or anti-chicken-IgY (Zymed); washed and signal detected using the Enhanced Chemiluminescence detection system as per manufacturers instructions (Amersham).

Rabbit anti-Ese1 antisera was raised against a peptide of the following sequence: MAQFPTPFGGSLDVWAITVEE. The antisera was affinity purified over the same peptide (Research Genetics). This peptide was also used at 5 µg to compete for the 5 µg of antibody per immunoprecipitation reaction. Chicken anti-Ese1 antisera was raised against a fusion protein between GST and amino acids 665-1213 of mouse Ese1. This sera was cleared of antibodies reacting against GST by incubation with glutathione s-transferase on glutathione agarose beads.

Immunofluorescence

For Immunofluorescent staining, Cos-1 cells were typically plated at a density of 2x10⁵ per 22x50mm coverglass and transfected with 2.5 µg of plasmid using Superfect (Qiagen Inc.). After 2 hours, the cells were washed with 10%FBS in Iscove's Media and fed with fresh 10%FBS in Iscove's Media. Two days later, these cultures were fixed with cold methanol for 30 minutes at room temperature. Cultures were washed three times 10 minutes with Phosphate buffered saline (PBS), blocked for one hour at room

temperature with 1%BSA in PBS and then incubated with primary antibody in blocking solution for one hour, also at room temperature. Slides were then washed three times 10 minutes with PBS, incubated with secondary antibody/1% BSA/PBS in the dark for one hour at room temperature. Finally, slides were washed three times 10 minutes in the dark
5 at room temperature and mounted using Dabco anti-fade solution (Sigma Chemical Co.). Slides were analyzed on the confocal microscope using the 63x objective and optical filters to separate signals on each channel.

For primary antibodies Mouse anti-myc monoclonal 9E10 (10 μ g/ml: Santa Cruz Biotech.), Rabbit anti-myc (5 μ g/ml: Upstate Biotechnology Inc.), Rabbit anti-Eps15
10 antisera #C20 (1 μ g/ml: Santa Cruz Biotech), Rabbit anti-Flag epitope antisera (5 μ g/ml: Zymed) and mouse anti-Dynamin I #D25520, which recognizes Dynamin in Cos-1 cells by both immunoprecipitation and western blotting (data not shown) (20 μ g/ml: Transduction labs, Inc.) were used. As secondary antibodies we used FITC-labelled goat anti-mouse (1:80 dilution) and Texas Red-labelled goat anti-rabbit antibodies (1:100 dilution)
15 (Jackson Immuno Research Laboratories Inc.).

References

1. H. Riezman, P. G. Woodman, G. van Meer, M. Marsh. Molecular mechanisms of Endocytosis. *Cell* 91: 731-738, 1997.
- 5 2. S. L. Schmid. Clathrin-coated vesicle formation and protein sorting: an integrated process. *Annu. Rev. Biochem.* 66: 511-548, 1997.

- 10 3. M. S. Robinson. Coats and budding vesicles. *Trends Cell. Biol.* 7: 99-102, 1997.
4. D. E. Warnock, S. L. Schmid. Dynamin GTPase, a force-generating molecular switch. *BioEssays* 18: 885-893, 1996.
- 15 5. R. Urrutia, J. R. Henley, T. Cook, M. A. McNiven. The dynamins: redundant or distinct functions for an expanding family of related GTPases? *Proc. Natl. Acad. Sci. USA* 94: 377-384, 1997.
6. P. Wigge, K. Kohler, Y. Vallis, C. A. Doyle, D. Owen, S. P. Hunt, H. T. McMahon. Amphiphysin Heterodimers: Potential Role in Clathrin-mediated Endocytosis.
- 20 *Molecular Biology of the Cell* 8: 2003-2015, 1997.
7. A. L. Munn, B. J. Stevenson, M. I. Geli, H. Riezman. end5, end6 and end7: mutations that cause actin delocalization and block the internalization step of endocytosis in *Saccharomyces cerevisiae*. *Molecular Biology of the Cell* 6: 1721-1742,
- 25 1995.
8. O. Shupliakov, P. Low, D. Grabs, H. Gad, H. Chen, C. David, K. Takei, P. De Camilli, L. Brodin. Synaptic vesicle endocytosis impaired by disruption of dynamin-SH3 domain interactions. *Science* 276: 259-263, 1997.
- 30 9. P. Wigge, Y. Vallis, H. T. McMahon. Inhibition of receptor-mediated endocytosis by the amphiphysin SH3 domain. *Current Biology* 7: 554-560, 1997.

10. H. McLauchlan, J. Newell, N. Morrice, A. Osborne, M. West, E. Smythe. A novel role for Rab5-GDI in ligand sequestration into clathrin-coated pits. *Current Biology* 8: 34-45, 1997.
- 5 11. P. J. Robinson, J.-M. Sontag, J.-P. Liu, E. M. Fykse, C. Slaughter, H. McMahon, T. C. Sudhof. Dynamin GTPase regulated by protein kinase C phosphorylation in nerve terminals. *Nature* 365: 163-166, 1993.

12. J.-P. Liu, A. T. R. Sim, P. J. Robinson. Calcineurin inhibition of Dynamin GTPase
10 activity coupled to nerve terminal depolarization. *Science* 265: 970-973, 1994.
13. A. Wilde, F. M. Brodsky. In vivo phosphorylation of Adaptors regulates their interaction with Clathrin. *The Journal of Cell Biology* 135: 635-645, 1996.
- 15 14. R. Bauerfeind, K. Takei, P. De Camilli. Amphiphysin I is associated with coated endocytic intermediates and undergoes stimulation-dependent dephosphorylation in nerve terminals. *J. Biol. Chem.* 272: 30984-30992, 1997.
- 20 15. S. van Delft, R. Govers, G. J. Strous, A. J. Verkleij, P. M. P. van Bergen en Henegouwen. Epidermal growth factor induces ubiquitination of Eps15. *Journal of Biological Chemistry* 272: 14013-14016, 1997.
16. J.-M. Galan, R. Haguenauer-Tsapis. Ubiquitin Lys63 is involved in ubiquitination of a membrane plasma membrane protein. *EMBO J* 16: 5847-5854, 1997.
- 25 17. J. Terrel, S. Shih, R. Dunn, L. Hicke. A function for monoubiquitination in the internalization of a G protein-coupled receptor. *Molecular Cell* 1: 193-202, 1998.
18. R. Govers, P. van Kerkhof, A. L. Schwartz, G. J. Strous. Linkage of the ubiquitin-
30 conjugating system and the endocytic pathway in ligand-induced internalization of the growth hormone receptor. *EMBO J.* 16: 4851-4858, 1997.
19. P. De Camilli, S. D. Emr, P. S. McPherson, P. Novick. Phosphoinositides as regulators in membrane traffic. *Science* 271: 1533-1539, 1996.

20. E. Kubler, H. Riezman. Actin and fimbrin are required for the internalization step of endocytosis in yeast. *EMBO J.* 12: 2855-2862, 1993.
- 5 21. H. Benedetti, S. Raths, F. Crausaz, H. Riezman. The END3 gene encodes a protein that is required for the internalization step of endocytosis and for actin cytoskeleton organization in yeast. *Mol. Biol. Cell.* 5: 1023-1037, 1994.

- 10 22. M. I. Geli, H. Riezman. Role of Type I Myosin in receptor-mediated endocytosis in yeast. *Science* 272: 533-535, 1996.
23. H.-Y. Tang, M. Cai. The EH-domain-containing protein Pan1 is required for normal organization of the actin cytoskeleton in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 16: 4897-4914, 1996.
- 15 24. V. Moreau, J.-M. Galan, G. Devilliers, R. Haguenauer-Tsapis, B. Winsor. The yeast Actin-related protein Arp2p is required for the internalization step of endocytosis. *Molecular Biology of the Cell* 8: 1361-1375, 1997.
- 20 25. B. Wendland, S. D. Emr. Pan1p, Yeast eps15, functions as a multivalent adaptor that coordinates protein-protein interactions essential for endocytosis. *Journal of Cell Biology* 141: 71-84, 1998.
- 25 26. F. Fazioli, L. Minichiello, B. Matoskova, W. T. Wong, P. P. Di Fiore. eps15, A novel tyrosine kinase substrate, exhibits transforming activity. *Mol. Cell. Biol.* 13: 5814-5828, 1993.
- 30 27. A. Benmerah, J. Gagnon, B. Begue, B. Megarbane, A. Dautry-Varsat, N. Cerf-Bensussan. The Tyrosine kinase substrate EPS15 is constitutively associated with the plasma membrane adaptor AP2. *J. Cell Biol.* 131: 1831-1838, 1995.
28. F. Tebar, T. Sorkina, A. Sorkin, M. Ericsson, T. Kirchhausen. Eps15 Is a component of Clathrin-coated Pits and Vesicles and is located at the Rim of Coated Pits. *Journal of Biological Chemistry* 271: 28727-28730, 1996.

29. F. Tebar, S. Confalonieri, R. E. Carter, P. P. Di Fiore, A. Sorkin. Eps15 is Constitutively Oligomerized due to Homophilic interaction of its Coiled-coil domain. *Journal of Biological Chemistry* 272: 15413-15418, 1997.

5

30. R. Carbone, S. Fre, G. Iannolo, F. Belleudi, M. P., P. G. Pelicci, M. R. Torrissi, P. P. Di Fiore. eps15 and eps15R are essential components of the endocytic pathway. *Cancer Research* 57: 5498-5504, 1997.

10 31. A. Benmerah, C. Lamaze, B. Begue, S. L. Schmid, A. Dautry-Varsat, N. Cerf-Bensussan. AP-2/Eps15 interaction is required for receptor-mediated endocytosis. *Journal of Cell Biology* 140: 1055-1062, 1998.

15 32. W. T. Wong, C. Schumacher, A. E. Salcini, A. Romano, P. Castagnino, P. G. Pelicci, P. P. Di Fiore. A protein-binding domain, EH, identified in the receptor tyrosine kinase substrate EPS15 and conserved in evolution. *Proc. Natl. Acad. Sci. USA* 92: 9530-9534, 1995.

20 33. P. P. Di Fiore, P. G. Pelicci, A. Sorkin. EH: a novel protein-protein interaction domain potentially involved in intracellular sorting. *Trends. Biochem. Sci.* 22: 411-413, 1997.

25 34. C. Schumacher, B. S. Knudsen, T. Ohuchi, P. P. Di Fiore, R. H. Glassman, H. Hanafusa. The SH3 domain of Crk binds specifically to a conserved proline-rich motif in Eps15 and Eps15R. *Journal of Biological Chemistry* 270: 15341-15347, 1995.

30 35. A. Benmerah, B. Begue, A. Dautry-Vasat, N. Cerf-Bensussan. The Ear of alpha-Adaptin interacts with the COOH-terminal domain of the EPS15 protein. *Journal of Biological Chemistry* 271: 12111-12116, 1996.

36. G. Iannolo, A. E. Salcini, I. Gaidarov, O. B. J. Goodman, J. Baulida, G. Carpenter, P. G. Pelicci, P. P. Di Fiore, J. H. Keen. Mapping of the molecular determinants involved in the interaction between Eps15 and AP2. *Cancer Research* 57: 240-245, 1997.

37. L. Coda, A. E. Salcini, S. Confalonieri, G. Pelicci, T. Sorkina, A. Sorkin, P. G. Pelicci, P. P. Di Fiore. Eps15R is a tyrosine kinase substrate with characteristics of a docking protein possibly involved in coated pits-mediated internalization. *Journal of Biological Chemistry* 273: 3003-3012, 1998.
38. B. Wendland, J. M. McCaffery, Q. Xiao, S. D. Emr. A Novel Fluorescence-activated Cell Sorter-based screen for yeast Endocytosis mutants identifies a yeast homologue of mammalian eps15. *J. Cell Biol.* 135: 1485-1500, 1996.
39. S. Raths, J. Rohrer, F. Crausaz, H. Riezman. end3 and end4: Two mutants defective in receptor-mediated and fluid-phase endocytosis in *Saccharomyces cerevisiae*. *The Journal of Cell Biology* 120: 55-65, 1993.
40. H. Y. Tang, A. Munn, M. Cai. EH domain proteins Pan1p and End3p are components of a complex that plays a dual role in organization of the cortical actin cytoskeleton and endocytosis in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 17: 4294-4304, 1997.
41. T. Zoladek, A. Tobiasz, G. Vaduva, M. Boguta, N. C. Martin, A. K. Hopper. MDP1, a *Saccharomyces cerevisiae* gene involved in mitochondrial/cytoplasmic protein distribution, is identical to the ubiquitin-protein ligase gene RSP5. *Genetics* 145: 595-603, 1997.
42. P. S. McPherson, E. P. Garcia, V. I. Slepnev, C. David, X. Zhang, D. Grabs, W. S. Sossin, R. Bauerfeind, Y. Nemoto, P. De Camilli. A presynaptic inositol-5-phosphatase. *Nature* 379: 353-357, 1996.
43. A. E. Salcini, S. Confalonieri, M. Doria, E. Santolini, E. Tassi, O. Minencova, G. Cesareni, P. G. Pelicci, P. P. Di Fiore. Binding specificity and in vivo targets of the EH domain, a novel protein-protein interaction module. *Genes & Development* 11: 2239-2249, 1997.

44. I. Gout, R. Dhand, I. D. Hiles, M. J. Fry, G. Panayotou, P. Das, O. Truong, N. F. Totty, J. Hsuan, G. W. Booker, I. D. Campbell, M. D. Waterfield. The GTPase dynamin binds to and is activated by a subset of SH3 domains. *Cell* 75: 25-36, 1993.
- 5 45. C. David, P. S. McPherson, O. Mundigl, P. De Camilli. A role of amphiphysin in synaptic vesicle endocytosis suggested by its binding to dynamin in nerve terminals. *Proc. Natl. Acad. Sci. USA* 93: 331-335, 1996.

46. M. H. Butler, C. David, G.-C. Ochoa, Z. Freyberg, L. Daniell, D. Grabs, O. Cremona, P. De Camilli. Amphiphysin II (SH3P9;BIN1), a member of the Amphiphysin/Rvs family, is concentrated in the cortical cytomatrix of axon initial segments and Nodes of Ranvier in brain and around T Tubules in skeletal muscle. *Journal of Cell Biology* 137: 1355-1367, 1997.
- 10 47. C. Leprince, F. Romero, D. Cussac, B. Vayssiere, R. Berger, A. Tavitian, J. H. Camonis. A new member of the Amphiphysin family connecting endocytosis and signal transduction pathways. *J. Biol. Chem.* 272: 15101-15105, 1997.
- 15 48. A. R. Ramjaun, K. D. Micheva, I. Bouchelet, P. S. McPherson. Identification and characterization of a nerve terminal-enriched amphiphysin isoform. *J. Biol. Chem.* 272: 16700-16706, 1997.
- 20 49. A. Wesp, L. Hicke, J. Palecek, R. Lombardi, T. Aust, A. L. Munn, H. Riezman. End4p/Sla2p Interacts with Actin-associated proteins for endocytosis in *Saccharomyces cerevisiae*. *Molecular Biology of the Cell* 8: 2291-2306, 1997.
- 25 50. E. de Heuvel, A. W. Bell, A. R. Ramjaun, K. Wong, W. S. Sossin, P. S. McPherson. Identification of the major Synaptojanin-binding proteins in the brain. *J. Biol. Chem.* 272: 8710-8716, 1997.
- 30 51. N. Ringstad, Y. Nemoto, P. De Camilli. The SH3p4/SH3p8/SH3p13 protein family: binding partners for synaptojanin and dynamin via a Grb2-like Src homology 3 domain. *Proc. Natl. Acad. Sci. USA* 94: 8569-8574, 1997.

52. Z. Wang, M. F. Moran. Requirement for the Adapter protein Grb2 in EGF receptor endocytosis. *Science* 272: 1935-1939, 1996.
- 5 53. A. B. Sparks, N. G. Hoffman, S. J. McConnel, D. M. Fowlkes, B. K. Kay. Cloning of ligand targets: Sytematic isolation of SH3 domain-containing proteins. *Nature Biotechnology* 14: 741-744, 1996.
-
54. P. Cupers, E. ter Haar, W. Boll, T. Kirchhausen. Parallel dimers and anti-parallel
10 tetramers formed by Epidermal Growth Factor Receptor Pathway Substrate Clone 15 (Eps15). *Journal of Biological Chemistry* 272: 33430-33434, 1997.
55. H. Stenmark, C. Bucci, M. Zerial. Expression of Rab GTPases using recombinant vaccinia virus. *Meth. Enzymol.* 257: 155-164, 1995.
- 15 56. S. van Delft, C. Schumacher, W. Hage, A. J. Verkleij, P. M. P. van Bergen en Henegouwen. Association and Colocalization of Eps15 with Adaptor Protein-2 and Clathrin. *The Journal of Cell Biology* 136: 811-821, 1997.
- 20 57. M. Toth, J. Grimsby, G. Buzsaki, G. P. Donovan. Epileptic seizures caused by inactivation of a novel gene, jerky, related to centromere binding protein-B in transgenic mice. *Nature Genetics* 11: 71-75, 1995.
58. J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning, a laboratory manual*.
25 *Second Edition*. (Cold Spring Harbor Press, Cold Spring Harbor, 1989).

Mouse Esei cDNA - Sequence ID NO:1

CGGCACGAGGAGGAGTGGAGCGGCGCGGGAGGGCGCGCAGCTTGGTTGCTCC
 GTAGTACGGCGGCTCGCAAGGGAGCATCCCGAGCGGGCTCCGGGACGGCCGG
 5 GAGGCAGGCAGGCGGGCGGGCGGGGATGGTGTGCGCGGCTGCGGACTCGGCG
 TTCTCGCGCGGCGGTGCGGGCTGCACTGATTTGTGTGAGGGGCGGCCGCGCGC
 ACCCGCCCGGAGATGAGGCGTCGATCAGCAAGGTGAACGTAATAGAACCATG
 GCTCAGTTTCCCACACCTTTCGGTGGTAGCCTGGATGTCTGGGCCATAACTGT
 GGAGGAAAGGGCCAAGCATGACCAGCAGTTCCTTAGCCTGAAGCCGATAGCG
 10 GGATTTATTACTGGTGATCAAGCGAGGAACCTTTTTTTTCCAATCTGGGTACCT
 CAGCCTGTCTTAGCACAAATATGGGCGCTAGCGGACATGAATAACGATGGAA
 GGATGGATCAAGTGGAATTTTCCATAGCCATGAAGCTTATCAAACCTGAAGCTA
 CAAGGATATCAGCTCCCCCTCCACACTTCCCCCTGTCATGAAACAGCAACCAGT
 GGCTATTTCAGTGCACCAGCATTTGGTATAGGAGGGATTGCTAGCATGCCAC
 15 CACTCACAGCTGTTGCTCCTGTGCCAATGGGCTCCATTCCAGTTGTTGGAATGT
 CTCCACCCTTAGTATCTTCTGTCCCTCCAGCAGCAGTGCCTCCCCTGGCTAACG
 GGGCTCCTCCCGTCATACAGCCTCTGCCTGCGTTTGCGCATCCTGCAGCCACAT
 GGCCAAAGAGTTCTTCCTTCAGCAGATCTGGTCCAGGGTCACAATTAACACT
 AAGTTACAGAAGGCACAATCATTGATGTGCGCCAGCGCCCCTCCAGCAGCAGA
 20 ATGGGCTGTGCCTCAGTCATCAAGGCTGAAATACAGGCAGTTATTCAACAGCC
 ACGACAAAACCTATGAGTGGACACTTAACAGGTCCCCAGGCAAGAACTATTCTC
 ATGCAATCAAGTTTACCCAGGCTCAGCTGGCTTCAATATGGAATCTTTCTGA
 CATTGATCAAGATGGAAAACCTACTGCAGAAGAATTTATCCTAGCTATGCACC
 TAATTGATGTTGCCATGTCTGGTCAGCCACTGCCGCCCGTCCTGCCTCCAGAAT
 25 ACATCCCTCCTTCCTTCAGAAGAGTTCGCTCCGGCAGTGGGATGTCCGTCATA
 AGCTCTTCTTCTGTGGATCAGAGGCTGCCTGAGGAGCCGTCGTCAGAGGATGA
 GCAGCAGCCAGAGAAGAAACTGCCTGTGACATTTGAAGATAAGAAGCGGGAG
 AACTTCGAGCGAGGCAGTGTGGAGCTGGAGAAGCGCCGCAAGCGCTCTTGG
 AGCAGCAGCGCAAAGAGCAGGAGCGGTTGGCTCAGCTGGAGCGCGCCGAGCA
 30 GGAGAGGAAAGAGCGGGAGCGCCAGGAGCAGGAGGCCAAGCGGCAGCTGGA
 GCTGGAGAAGCAGCTGGAGAAGCAGCGGGAGCTGGAGCGGCAGCGAGAGGA
 GGAGAGGAGGAAGGAGATCGAGAGGCGCGAGGCCGAAAACGGGAACCTGGA
 AAGGCAGCGACAACCTTGAATGGGAACGGAACCGGAGACAGGAACCTCTGAAT
 CAGAGGAACAAGGAGCAGGAGGGCACCGTGGTCCTGAAGGCAAGGAGGAAG

ACTCTGGAGTTTGAGTTAGAAGCTCTGAATGACAAAAAGCATCAGCTAGAAGG
 AAAACTTCAGGATATCAGGTGTCGACTGGCAACCCAGAGGCAAGAAATTGAG
 AGCACGAACAAGTCTAGAGAGCTAAGAATTGCTGAAATCACCCACTTACAGCA
 GCAGTTGCAGGAATCTCAGCAAATGCTTGGAAGACTTATTCAGAGAAACAGA
 5 TACTCAGTGACCAGTTAAAACAAGTCCAGCAGAACAGTTTGCATAGAGACTCG
 CTTCTTACCCTCAAAGAGCCTTGGAAGCAAAGGAGCTGGCCCGGCAGCAGCT
 CCGGGAGCAGCTGGACGAGGTGGAGAGAGAGACCAGGTCAAAGCTGCAGGA
 GATTGATGTTTTCAACAACCAGCTGAAGGAACTGAGAGAGATACATAGCAAAC
 AGCAACTCCAGAAGCAGAGGTCCCTGGAGGCAGCGCGACTGAAGCAGAAAGA
 10 GCAGGAGAGGAAGAGCCTGGAGTTAGAGAAGCAAAAGGAAGACGCTCAGAG
 ACGAGTTCAGGAAAGGGACAAGCAATGGCTGGAGCATGTGCAGCAGGAGGAG
 CAGCCACGCCCCCGGAAACCCACGAGGAGGACAGACTGAAGAGGGAAGACA
 GTGTCAGGAAGAAGGAGGCGGAAGAGAGAGCCAAGCCGGAAATGCAAGACA
 AGCAGAGTCGGCTTTTCCATCCGCATCAGGAGCCAGCTAAGCTGGCCACCCAG
 15 GCACCCTGGTCTACCACAGAGAAAGGCCCGCTTACCATTTCTGCACAGGAGAG
 TGTAAGAGTGGTATATTACCGAGCGCTGTACCCCTTTGAATCCAGAAGTCACG
 ATGAGATCACCATCCAGCCAGGAGATATAGTCATGGTGGATGAAAGCCAGAC
 TGGAGAGCCAGGATGGCTTGGAGGAGAGCTGAAAGGGAAGACGGGATGGTTC
 CCTGCAAACCTATGCAGAAAAGATTCCAGAAAATGAGGTTCCCACTCCAGCCAA
 20 ACCAGTGACCGATCTGACATCTGCCCTGCCCCCAAACCTGGCTCTGCGTGAGA
 CCCCTGCTCCTTTGCCAGTGACCTCTTCTGAGCCCTCCACAACCCCCAACAAC
 GGGCAGACTTCAGTTCCACGTGGCCCAGCAGCTCAAACGAGAAGCCAGAAAC
 GGACAACTGGGATACGTGGGCGGCTCAGCCTTCTCTGACCGTACCTAGTGCTG
 GCCAGTTACGGCAGAGATCAGCCTTTACCCAGCCACAGCCACTGGCTCCTCC
 25 CCATCTCCCGTCCTGGGCCAGGGTGAAAAGGTGGAAGGGCTACAAGCGCAAG
 CCCTGTATCCCTGGAGAGCCAAAAAGACAACCACTTAAATTTTAACAAAAGT
 GACGTCATCACCGTTCTGGAACAGCAAGACATGTGGTGGTTTGGAGAAGTTCA
 AGGTCAGAAGGGTTGGTTCCCAAGTCTTACGTGAAACTCATTTACAGGGCCCCG
 TAAGGAAATCCACAAGCATCGATACTGGCCCTACTGAAAGTCCTGCTAGTCTA
 30 AAGAGAGTGGCTTCCCCGGCCGCCAAGCCAGCCATTCCCGGAGAAGAGTTTAT
 TGCCATGTACACATACGAGAGTTCTGAGCAAGGAGATTTAACCTTTACAGCAAG
 GGGATGTGATTGTGGTTACCAAGAAAGATGGTGAAGTGGTGGACGGGAACGGT
 GGGCGACAAGTCCCGAGTCTTCCCTTCTAACTATGTGAGGCTTAAAGATTCAG
 AGGGCTCTGGAAGTCTGGGAAAACAGGGAGTTTAGGAAAAAACCTGAAAT

TGCCCAGGTTATTGCTTCCTACGCTGCTACTGGTCCCGAACAACCTCACCCCTGGC
 TCCTGGGCAGCTGATTCTGATCCGGAAAAAGAACCCAGGTGGATGGTGGGAA
 GGAGAACTGCAAGCTCGAGGGAAAAAGCGCCAGATAGGGTGGTTTCCAGCAA
 ATTATGTCAAACCTTCTAAGCCCCGGAACAAGCAAAATCACCCCAACTGAGCTA
 5 CCCAAGACCGCAGTGCAGCCAGCAGTGTGCCAGGTGATCGGGATGTACGATT
 ACACCGCCCAGAACGATGACGAACTAGCCTTCAGCAAAGGCCAGATCATCAAC
 GTCCTCAACAAGGAGGACCCGGACTGGTGGAAAGGAGAAGTCAGTGGGCAAG
 TTGGGCTCTTCCCATCCAATTATGTAAAGCTGACCACAGACATGGACCCCAGC
 CAGCAATGAATCATATGTTGTCCATCCCCCCTCAGGCTTGAAAGTCCTCAAA
 10 GAGACCCACTATCCCATATCACTGCCCAGAGGGATGATGGGAGATGCAGCCTT
 GATCATGTGACTTGCAGCATGATCACCTACTGCCTTCTGAGTAGAAGAACTCA
 CTGCAGAGCAGTTTACCTCATTTGACCTTAGTTGCATGTGATCGAAATGTCTG
 AGTCACTGCGTGCAGAGGCAGAAGCAAATTGCAGAACTGCACAGGGTGGTGG
 GTCCTTTTGGGGCTTTCCTAGTCACTCAGACTGACCGGCCCCCGCCTTCACACG
 15 GCGCCTTTCAATAGTTTTAAGATTATTTTTAAATGTGTATTTTAGCCTTTTAAT
 AAAAATCTCAATCAATTACTTCTTTGCCTATTTTGGTTTTACAAAAACACCCAC
 TATCAAGGAGTGCCTGTCTGCGGACGATTAAAATGCTGTTCCGGGCGTACCGT
 AAAGTGAAGAGCTTGCTGTACCTTTGCCGTTTGTCCAGTGTTCCCAACCACATTG
 TGTAGTTTGGGGCTGTTCCCTGCCGTAGAGCACAGAGGAGATGGGTGTACCTG
 20 TTTTGAAGATGTGTATGTAGACTGAGCCTGACTATGGAAGGGGTTATGCTTGT
 CTGTGACCATCACGTGTACCTGTGCGCATGTACCATCTGTACCGAAGAAGTA
 GCTCTTCCTCCATGGCTAAACCCACCACCGTGTACAGTGCTCTCATCTACTGCA
 TTCATTTTACTTTGCACAGTGACCTTGTAGCCACCTGAGGAAGCACCCATGTTT
 CCGTTTGGTCTCAGATGTACCTAGTTGTGCCCGTGTTTTGTTTTTATTTTTCAA
 25 TCTGGCATGTCTTCACACCATAAACTAGTAAGACGCCAACTGCCCAGGCGGTT
 ACGATCATCAGTACCCACCGTCTTAGTCTCTGTTACGTGAAGTTTATTCCAGTT
 GCTTTTTATGGAATATCTTGAACAAGTAATCTTCTTGACAAGAAAGAATGTAT
 AGAAGTCTCCCTGCAATTAATTTCCAGTGTTTACATTTTTTAACTAGACTGTG
 GGGGTTGCTACAGATTAATATGAAATGGCGCTCCTGGTCCGTGTGTGTGTAA
 30 CTTGTGCTGTAGCTGAAGCCGTGTGTCCTTAGATATTAGTTGGAAGTCGGGAA
 GAGAATTCGATATCAAGCTT

Mouse Ese1 coding sequence - Sequence ID NO:2

ATGGCTCAGTTTCCCACACCTTTCGGTGGTAGCCTGGATGTCTGGGCCATAAC
 5 TGTGGAGGAAAGGGCCAAGCATGACCAGCAGTTCCTTAGCCTGAAGCCGATA
 GCGGGATTTATTACTGGTGATCAAGCGAGGAACTTTTTTTTCCAATCTGGGT
 ACCTCAGCCTGTCTTAGCACAAATATGGGCGCTAGCGGACATGAATAACGATG
 GAAGGATGGATCAAGTGGAATTTTCCATAGCCATGAAGCTTATCAAACCTGAAG
 CTACAAGGATATCAGCTCCCCTCCACACTTCCCCCTGTCATGAAACAGCAACC
 10 AGTGGCTATTTCCAGTGCACCAGCATTTGGTATAGGAGGGATTGCTAGCATGC
 CACCACTCACAGCTGTTGCTCCTGTGCCAATGGGCTCCATTCCAGTTGTTGGA
 ATGTCTCCACCTTAGTATCTTCTGTCCCTCCAGCAGCAGTGCCTCCCCTGGCT
 AACGGGGCTCCTCCCGTCATACAGCCTCTGCCTGCGTTTGCGCATCCTGCAGC
 CACATGGCCAAAGAGTTCTTCCTTCAGCAGATCTGGTCCAGGGTCACAATTAA
 15 AACTAAGTTACAGAAGGCACAATCATTCGATGTCGCCAGCGCCCCTCCAGCA
 GCAGAATGGGCTGTGCCTCAGTCATCAAGGCTGAAATACAGGCAGTTATTCAA
 CAGCCACGACAAAACCTATGAGTGGACACTTAACAGGTCCCCAGGCAAGAACT
 ATTCTCATGCAATCAAGTTTACCCCAGGCTCAGCTGGCTTCAATATGGAATCTT
 TCTGACATTGATCAAGATGGAAAACCTCACTGCAGAAGAATTTATCCTAGCTAT
 20 GCACCTAATTGATGTTGCCATGTCTGGTCAGCCACTGCCGCCCCGTCCTGCCTCC
 AGAATACATCCCTCCTTCCTTCAGAAGAGTTCGCTCCGGCAGTGGGATGTCCG
 TCATAAGCTCTTCTTCTGTGGATCAGAGGCTGCCTGAGGAGCCGTCGTCAGAG
 GATGAGCAGCAGCCAGAGAAGAACTGCCTGTGACATTTGAAGATAAGAAGC
 GGGAGAACTTCGAGCGAGGCAGTGTGGAGCTGGAGAAGCGCCGCCAAGCGCT
 25 CTTGGAGCAGCAGCGCAAAGAGCAGGAGCGGTTGGCTCAGCTGGAGCGCGCC
 GAGCAGGAGAGGAAAGAGCGGGAGCGCCAGGAGCAGGAGGCCAAGCGGCAG
 CTGGAGCTGGAGAAGCAGCTGGAGAAGCAGCGGGAGCTGGAGCGGCAGCGA
 GAGGAGGAGAGGAGGAAGGAGATCGAGAGGCGCGAGGCCGCAAAACGGGAA
 CTGGAAAGGCAGCGACAACCTGAATGGGAACGGAACCGGAGACAGGAACTCC
 30 TGAATCAGAGGAACAAGGAGCAGGAGGGCACCGTGGTCCTGAAGGCAAGGAG
 GAAGACTCTGGAGTTTGAGTTAGAAGCTCTGAATGACAAAAGCATCAGCTAG
 AAGGAAAACCTCAGGATATCAGGTGTGACTGGCAACCCAGAGGCAAGAAAT
 TGAGAGCACGAACAAGTCTAGAGAGCTAAGAATTGCTGAAATCACCCACTTAC
 AGCAGCAGTTGCAGGAATCTCAGCAAATGCTTGGAAGACTTATTCCAGAGAAA

CAGATACTCAGTGACCAGTTAAAACAAGTCCAGCAGAACAGTTTGCATAGAGA
 CTCGCTTCTTACCCTCAAAAGAGCCTTGGAAAGCAAAGGAGCTGGCCCCGGCAGC
 AGCTCCGGGAGCAGCTGGACGAGGTGGAGAGAGAGACCAGGTCAAAGCTGCA
 GGAGATTGATGTTTTCAACAACCAGCTGAAGGAACTGAGAGAGATACATAGC
 5 AAACAGCAACTCCAGAAGCAGAGGTCCCTGGAGGCAGCGGACTGAAGCAGA
 AAGAGCAGGAGAGGAAGAGCCTGGAGTTAGAGAAGCAAAAGGAAGACGCTC
 AGAGACGAGTTCAGGAAAGGGACAAGCAATGGCTGGAGCATGTGCAGCAGGA
 GGAGCAGCCACGCCCCCGGAAACCCACGAGGAGGACAGACTGAAGAGGGAA
 GACAGTGTCAGGAAGAAGGAGGCGGAAGAGAGAGCCAAGCCGGAAATGCAA
 10 GACAAGCAGAGTCGGCTTTTCCATCCGCATCAGGAGCCAGCTAAGCTGGCCAC
 CCAGGCACCCTGGTCTACCACAGAGAAAGGCCCGCTTACCATTTCTGCACAGG
 AGAGTGTAAGAGTGGTATATTACCGAGCGCTGTACCCCTTTGAATCCAGAAGT
 CACGATGAGATCACCATCCAGCCAGGAGATATAGTCATGGTGGATGAAAGCC
 AGACTGGAGAGCCAGGATGGCTTGGAGGAGAGCTGAAAGGGAAGACGGGAT
 15 GGTTCCCTGCAAACATATGCAGAAAAGATTCCAGAAAATGAGGTTCCCACTCCA
 GCCAAACCAGTGACCGATCTGACATCTGCCCTTGCCCCAAACTGGCTCTGCG
 TGAGACCCCTGCTCCTTTGCCAGTGACCTCTTCTGAGCCCTCCACAACCCCCAA
 CAACTGGGCAGACTTCAGTTCCACGTGGCCCAGCAGCTCAAACGAGAAGCCA
 GAAACGGACAACCTGGGATACGTGGGCGGCTCAGCCTTCTCTGACCGTACCTAG
 20 TGCTGGCCAGTTACGGCAGAGATCAGCCTTTACCCCAGCCACAGCCACTGGCT
 CCTCCCCATCTCCCGTCCTGGGCCAGGGTGAAAAGGTGGAAGGGCTACAAGCG
 CAAGCCCTGTATCCCTGGAGAGCCAAAAAAGACAACCACTTAAATTTTAACAA
 AAGTGACGTCATCACCGTTCTGGAACAGCAAGACATGTGGTGGTTTGGAGAAG
 TTCAAGGTCAGAAGGGTTGGTTCCCCAAGTCTTACGTGAAACTCATTTAGGG
 25 CCCGTAAGGAAATCCACAAGCATCGATACTGGCCCTACTGAAAGTCCTGCTAG
 TCTAAAGAGAGTGGCTTCCCCGGCCGCAAGCCAGCCATTCCCGGAGAAGAGT
 TTATTGCCATGTACACATACGAGAGTTCTGAGCAAGGAGATTTAACCTTTCAG
 CAAGGGGATGTGATTGTGGTTACCAAGAAAGATGGTGACTGGTGGACGGGAA
 CGGTGGGCGACAAGTCCGGAGTCTTCCCTTCTAACTATGTGAGGCTTAAAGAT
 30 TCAGAGGGCTCTGGAACCTGCTGGGAAAACAGGGAGTTTAGGAAAAAACCTG
 AAATTGCCCAGGTTATTGCTTCTACGCTGCTACTGGTCCCGAACAACCTCACCC
 TGGCTCCTGGGCAGCTGATTCTGATCCGGAAAAAGAACCCAGGTGGATGGTG
 GGAAGGAGAACTGCAAGCTCGAGGGAAAAAGCGCCAGATAGGGTGGTTTCCA
 GCAAATTATGTCAAACCTTCTAAGCCCCGGAACAAGCAAAATCACCCCAACTGA

GCTACCCAAGACCGCAGTGCAGCCAGCAGTGTGCCAGGTGATCGGGATGTAC
 GATTACACCGCCCAGAACGATGACGAAGTACCTTCAGCAAAGGCCAGATCAT
 CAACGTCCTCAACAAGGAGGACCCGGACTGGTGGAAAGGAGAAGTCAGTGGG
 CAAGTTGGGCTCTTCCCATCCAATTATGTAAAGCTGACCACAGACATGGACCC
 5 CAGCCAGCAATGAATCATATGTTGTCCATCCCCCCTCAGGCTTGAAAGTCCT
 CAAAGAGACCCACTATCCCATATCACTGCCCAGAGGGATGA

Mouse Esei protein - Sequence ID NO:3

10 MAQFPTPFGGSLDVWAITVEERAKHDQQFLSLKPIAGFITGDQARNFFFQSGLPQP
 VLAQIWALADMNNDGRMDQVEFSIAMKLIKLLQGYQLPSTLPPVMKQQPVAIS
 SAPAFGIGGIASMPPLTAVAPVPMGSIPVVGMSPPLVSSVPPAAVPPLANGAPPVIQ
 PLPAFAHPAATWPKSSSFSGPGSQLNTKLQKAQSFDVASAPPAAEWAVPQSSR
 LKYRQLFNHDKTMSGHLTGPQARTILMQSSLPQAQLASIWNLSDDIDQDGKLTAE
 15 EFILAMHLIDVAMSGQPLPPVLPPEYIPPSFRRVRSGSGMSVISSSSVDQRLPEEPSS
 EDEQQPEKKLPVTFEDKKRENFERGSVELEKRRQALLEQQRKEQERLAQLERAEO
 ERKERERQEQAQRQLELEKQLEKQRELERQREEERRKEIERREAARKRELERQRQ
 LEWERNRRQELLNQRNKEQEGTVVLKARRKTLEFELEALNDKKHQLEGLQDIR
 CRLATQRQEIESTNKSRELRIAEITHLQQQLQESQQMLGRLIPEKQILSDQLKQVQ
 20 QNSLHRDSSLTLKRALEAKELARQQLREQLDEVERETRSLQLEIDVFNNQLKELR
 EIIHSKQQLQKQRSLEAARLKQKEQERKSLELEKQKEDAQRRVQERDKQWLEHVQ
 QEEQPRPRKPHEEDRLKREDSVRKKEAEERAKPEMQDKQSRLFHPHQEPAKLAT
 QAPWSTTEKGPLTISAQESVKVVYYRALYPFESRSHDEITIQPGDIVMVDESQTGE
 PGWLGGELKGKTGWFPANYAEKIPENEVPTPAKPVTDLTSAPAPKLALRETPAPL
 25 PVTSSSEPSTTPNNWADFSSTWPSSSNEKPETDNWDTWAAQPSLTVPSAGQLRQRS
 AFTPATATGSSPSPVLGQGEKVEGLQAQALYPWRAKKDNHLNFNKSDVITVLEQ
 QDMWWFGEVQGQKGWFPKSYVKLISGPVRKSTSIDTGPTESPASLKRVASPAAKP
 AIPGEEFIAMYTYESSEQGDLTQQGDVIVVTKDGDWWTGTVDKSGVFPSNY
 VRLKDSESGGTAGKTGSLGKKPEIAQVIASAAATGPEQLTLAPGQLILIRKKNPGG
 30 WWEGELQARGKKRQIGWFPANYVKLLSPGTSKITPTE
 LPKTAVQPAVCQVIGMYDYTAQNDELAFSGQIINVLNKEDPDWWKGEVSGQ
 VGLFPSNYVKLTTDMDPSQQ

Mouse Ese2 cDNA - Sequence ID NO:4

CCCTTCCTTTCTTTTTTTGTGTTTCGCCTTCGGCCGTGCCGGCTGAGAGCCCAG
5 CAGCCGTGACAGGCTGCGCAACAGGTTTCGCTGCGGCCGGCCTGACGACTGAC
CCGGCGGCGGCGGCCGCGGCACGGCAGGGTCTTCCCGGAGCTTGGCCGCGCC
CACGCGCCGGTGTCTGAGGAGCGCGCGGGGTTCGCGCCGGGACGTGCGCGAGGC
GCCAGATGGCTGAGAGCTGCAAGAAGAAGTCAGGATCATGATGGCTCAGTTT

CCCACAGCGATGAATGGAGGGCCAAATATGTGGGCTATTACATCTGAAGAAC
10 GTACTAAGCATGATAAACAGTTTGATAACCTCAAACCTTCAGGAGGTTACATA
ACAGGTGATCAAGCCCGTACTTTTTTCTACAGTCAGGTCTGCCGGCCCCGGT
TTTAGCTGAAATATGGGCCTTATCAGATCTGAACAAGGATGGGAAGATGGACC
AGCAAGAGTTCTCTATAGCTATGAAACTCATCAAGTTAAAGTTGCAGGGCCAA
CAGCTGCCTGTAGTCCTCCCTCCTATCATGAAACAACCCCTATGTTCTCTCCA
15 CTAATCTCTGCTCGTTTTTGGGATGGGAAGCATGCCCAATCTGTCCATTCATCAG
CCATTGCCTCCAGTTGCACCTATAGCAACACCCTTGTCTTCTGCTACGTCAGGG
ACCAGTATTCCTCCCCTAATGATGCCTGCTCCCCTAGTGCCTTCTGTTAGTACA
TCCTCATTACCAAATGGAAGTGCAGTCTCATTACGCCTTTATCCATTCCCTTAT
TCTTCTTCAACATTGCCTCATGCATCATCTTACAGCCTGATGATGGGAGGATTT
20 GGTGGTGCTAGTATCCAGAAGGCCCAGTCTCTGATTGATTTAGGATCTAGTAG
CTCAACTTCCTCAACTGCTTCCCTCTCAGGGAACCTCACCTAAGACAGGGACCT
CAGAGTGGGCAGTTCCTCAGCCTTCAAGATTAAAGTATCGGCAAAAATTTAAT
AGTCTAGACAAAGGCATGAGCGGATACCTCTCAGGTTTTCAAGCTAGAAATGC
CCTTCTTCAGTCAAATCTCTCTCAAACCTCAGCTAGCTACTATTTGGACTCTGGC
25 TGACATCGATGGTGACGGACAGTTGAAAGCTGAAGAATTTATTCTGGCGATGC
ACCTCACTGACATGGCCAAAGCTGGACAGCCACTACCACTGACGTTGCCTCCC
GAGCTTGTCCCTCCATCTTTTCAGAGGGGGAAAGCAAGTTGATTCTGTTAATGG
AACTCTGCCTTCATATCAGAAAACACAAGAAGAAGAGCCTCAGAAGAACTGC
CAGTTACTTTTGAGGACAAACGGAAAGCCAACCTATGAACGAGGAAACATGGA
30 GCTGGAGAAGCGACGCCAAGTGTTGATGGAGCAGCAGCAGAGGGAGGCTGAA
CGCAAAGCCCAGAAAGAGAAGGAAGAGTGGGAGCGGAAACAGAGAGAACTG
CAAGAGCAAGAATGGAAGAAGCAGCTGGAGTTGGAGAAACGCTTGGAGAAAC
AGAGAGAGCTGGAGAGACAGCGGGAGGAAGAGAGGAGAAAGGAGATAGAAA
GACGAGAGGCAGCAAAACAGGAGCTTGAGAGACAACGCCGTTTAGAATGGGA

AAGACTCCGTCGGCAGGAGCTGCTCAGTCAGAAGACCAGGGAACAAGAAGAC
 ATTGTCAGGCTGAGCTCCAGAAAGAAAAGTCTCCACCTGGAAGTGGAAAGCAGT
 GAATGGAAAACATCAGCAGATCTCAGGCAGACTACAAGATGTCCAAATCAGA
 AAGCAAACACAAAAGACTGAGCTAGAAGTTTTGGATAAACAGTGTGACCTGG
 5 AAATTATGGAAATCAAACAACCTTCAACAAGAGCTTAAGGAATATCAAAATAAG
 CTTATCTATCTGGTCCCTGAGAAGCAGCTATTAAACGAAAGAATTAAAAACAT
 GCAGCTCAGTAACACACCTGATTACAGGGATCAGTTTACTTCATAAAAAGTCAT
 CAGAAAAGGAAGAATTATGCCAAAGACTTAAAGAACAATTAGATGCTCTTGA
 AAAAGAACTGCATCTAAGCTCTCAGAAATGGATTCAATTAACAATCAGCTGA
 10 AGGAACTCAGAGAAAGCTATAATACACAGCAGTTAGCCCTTGAACAACCTTCAT
 AAAATCAAACGTGACAAATTGAAGGAAATCGAAAGAAAAAGATTAGAGCAAA
 TTCAAAAAAAGAACTAGAAGATGAGGCTGCAAGGAAAGCAAAGCAAGGAAA
 AGAAAACCTTGTGGAGAGAAAAGTATTAGAAAGGAAGAAGAGGAAAAGCAAAA
 ACGACTCCAGGAAGAAAAGTCACAGGACAAAACCTCAAGAAGAGGAACGAAAA
 15 GCTGAGGCAAAACAAAGTGAGACAGCCAGTGCTTTGGTGAATTACAGAGCAC
 TGTACCCTTTTGAAGCAAGAAACCATGATGAGATGAGTTTTAGTTCTGGGGAT
 ATAATTCAGGTTGATGAAAAAACTGTAGGAGAGCCTGGTTGGCTTTATGGTAG
 TTTTCAGGGAAAGTTTGGCTGGTTCCCTGCAACTATGTAGAAAAAGTGCTGT
 CAAGTGAAAAAGCTCTGTCTCCTAAGAAGGCCTTACTTCCTCCTACAGTGTCT
 20 CTCTCTGCTACCTCAACTTCTTCCCAGCCACCAGCATCAGTGACTGATTATCAC
 AATGTATCCTTCTCAAACCTTACTGTTAATACAACATGGCAGCAGAAGTCAGC
 TTTTACCCGCACTGTGTCCCCTGGATCTGTGTCCCCCATTCACGGACAGGGGC
 AGGCTGTAGAAAACCTGAAAGCCCAGGCCCTTTGTTCCCTGGACGGCAAAGAA
 GGAGAACCACCTGAACTTCTCAAAGCACGACGTCATCACTGTCCTGGAGCAGC
 25 AGGAAAACCTGGTGGTTTGGGGAGGTGCACGGAGGAAGAGGATGGTTCCCCAA
 GTCTTATGTCAAGCTCATTCTGGGAATGAAGTACAGCGAGGAGAGCCAGAA
 GCTTTGTATGCAGCTGTGACTAAGAAACCTACCTCCACAGCCTATCCAGTTAC
 CTCCACAGCCTATCCAGTTGGAGAAGACTACATTGCACTTTATTCATACTCAA
 GTGTAGAGCCCGGGGATTTGACTTTCACTGAAGGTGAAGAAATTCTAGTGACC
 30 CAGAAAGATGGAGAGTGGTGGACAGGAAGTATTGGAGAGAGAACTGGAATCT
 TCCCGTCCAACTACGTCAGACCAAAGGATCAAGAGAATTTTGGGAATGCTAGC
 AAATCTGGAGCATCAAACAAAAAACCCGAGATCGCTCAAGTAACTTCAGCATA
 TGCTGCTTCAGGGACTGAGCAGCTCAGCCTTGCGCCAGGACAGTTAATATTAA
 TCTTAAAGAAAAACACAAGCGGGTGGTGGCAAGGAGAGCTACAGGCCAGAGG

GAAGAAACGACAGAAGGGATGGTTTCCTGCCAGCCATGTAAAGCTGCTAGGT
 CCAAGCAGTGAAAGAACCATGCCTACTTTTCACGCTGTATGTCAAGTGATTGC
 TATGTATGACTACATGGCGAATAACGAAGATGAGCTCAATTTCTCAAAGGAC
 AGCTGATTAATGTTATGAACAAAGATGACCCTGACTGGTGGCAAGGAGAAAC
 5 CAATGGTCTGACTGGTCTCTTTTCCTTCAAACATATGTTAAGATGACAACAGACTC
 AGATCCAAGTCAACAGTGGTGTGCTGACCTCCAAGCCCTGGACACAATGCAGC
 CTACGGAGAGGAAGCGACAGGGCTACATTCACGAGCTCATTGAGACAGAGGA
 GCGGTACATGGACGACCTGCAACTTTTTGAACAAAAAACTCTCCTTTGAGGGC
 CTGGGGAAGCCAGAACCAGGGGAGCTGCCCACAAGGCTGGGTCTAAAGACAG
 10 ATTTTGCTCTCCAGGACAGAGGAGCATCACATCGGCTTCATCCATCCAAACA
 AGCCACACTCGCTGGGCCTGGTATTTTATTGCACCACTAAAATTGCTAGCAAT
 CTATGCAAACATGATCTTTTTAAACAAACGCCACAGCACAGTGCCTTGTACTA
 GTGTTAACCTGTTGAGCTGTGTTAGATGCCAGGGTTTCCATTTTCAGGGCTATA
 AAAGTATTATGTGGGAAATGAGACATCAGACCACCGGACGTTACCACTTGGCA
 15 AATCTGTCCACTGTGGAGTTGGTGTGTTGGAACCATTCCACACTATGTGACC
 TCTGCTGGGTCACACACTCAGGAGGTGAAGGGCTGAGATGAAATGCTGCAGC
 CTTGGGGCTTGTGCAGCCTGATACTGAAATAGCATCCACTTGTGCACTGAATA
 AATAGAACTTGATCGTTTTATTCTGACTAGATATTATCATTCTCTGCTAAGAC
 AATATAGTTTGAAATATTATAGTTTGAATATAAGGAGGAAAGCTTGATGTACT
 20 TTAAATATACTGTGAACTCTAATAATGTGGGGATATTTTTCACTTTAATTTTC
 TTAAGTATAAATTATTTATGTAAATTCTTTGTTTTGCATATTTTCATAGAACATG
 CATCTTTAAGCTTTATCATTGCCAACAATGTACAGAAAGAGAATAAAAGTATA
 AGTTTATGAATGTAAAAAAAAAAAAAAAAAAAAA

25 Mouse Ese2 coding sequence - Sequence ID NO:5

ATGGCTCAGTTTCCCACAGCGATGAATGGAGGGCCAAATATGTGGGCTATTAC
 ATCTGAAGAACGTACTAAGCATGATAAACAGTTTGATAACCTCAAACCTTCAG
 GAGGTTACATAACAGGTGATCAAGCCCGTACTTTTTTCCTACAGTCAGGTCTG
 30 CCGGCCCGGTTTTAGCTGAAATATGGGCCTTATCAGATCTGAACAAGGATGG
 GAAGATGGACCAGCAAGAGTTCTCTATAGCTATGAACTCATCAAGTTAAAGT
 TGCAGGGCCAACAGCTGCCTGTAGTCCTCCCTCCTATCATGAAACAACCCCT
 ATGTTCTCTCCACTAATCTCTGCTCGTTTTGGGATGGGAAGCATGCCCAATCTG
 TCCATTCATCAGCCATTGCCTCCAGTTGCACCTATAGCAACACCCTTGTCTTCT

GCTACGTCAGGGACCAGTATTCCTCCCCTAATGATGCCTGCTCCCCTAGTGCCT
 TCTGTTAGTACATCCTCATTACCAAATGGAAGTCCAGTCTCATTACGCCTTTA
 TCCATTCCCTTATTCTTCTTCAACATTGCCTCATGCATCATCTTACAGCCTGATG
 ATGGGAGGATTTGGTGGTGCTAGTATCCAGAAGGCCAGTCTCTGATTGATTT
 5 AGGATCTAGTAGCTCAACTTCCTCAACTGCTTCCCTCTCAGGGAAGTCACTAA
 GACAGGGACCTCAGAGTGGGCAGTTCCTCAGCCTTCAAGATTAAAGTATCGGC
 AAAAATTTAATAGTCTAGACAAAGGCATGAGCGGATACCTCTCAGGTTTTCAA
 GCTAGAAATGCCCTTCTTCAGTCAAATCTCTCTCAAAGTCAAGTACTATT
 TGGACTCTGGCTGACATCGATGGTGACGGACAGTTGAAAGCTGAAGAATTTAT
 10 TCTGGCGATGCACCTCACTGACATGGCCAAAGCTGGACAGCCACTACCACTGA
 CGTTGCCTCCCGAGCTTGTCCCTCCATCTTTTCAGAGGGGGAAAGCAAGTTGAT
 TCTGTTAATGGAAGTCTGCCTTCATATCAGAAAACACAAGAAGAAGAGCCTCA
 GAAGAACTGCCAGTTACTTTTGAGGACAAACGGAAAGCCAACTATGAACGA
 GGAAACATGGAGCTGGAGAAGCGACGCCAAGTGTTGATGGAGCAGCAGCAGA
 15 GGGAGGCTGAACGCAAAGCCCAGAAAGAGAAGGAAGAGTGGGAGCGGAAAC
 AGAGAGAACTGCAAGAGCAAGAATGGAAGAAGCAGCTGGAGTTGGAGAAAC
 GCTTGGAGAAACAGAGAGAGCTGGAGAGACAGCGGGAGGAAGAGAGGAGAA
 AGGAGATAGAAAGACGAGAGGCAGCAAAACAGGAGCTTGAGAGACAACGCC
 GTTTAGAATGGGAAAGACTCCGTCGGCAGGAGCTGCTCAGTCAGAAGACCAG
 20 GGAACAAGAAGACATTGTCAGGCTGAGCTCCAGAAAGAAAAGTCTCCACCTG
 GAACTGGAAGCAGTGAATGGAAAACATCAGCAGATCTCAGGCAGACTACAAG
 ATGTCCAAATCAGAAAGCAAACACAAAAGACTGAGCTAGAAGTTTTGGATAA
 ACAGTGTGACCTGGAAATTATGGAAATCAAACAAGTCAACAAGAGCTTAAGG
 AATATCAAAATAAGCTTATCTATCTGGTCCCTGAGAAGCAGCTATTAAACGAA
 25 AGAATTA AAAACATGCAGCTCAGTAACACACCTGATTCAGGGATCAGTTTACT
 TCATAAAAAGTCATCAGAAAAGGAAGAATTATGCCAAAGACTTAAGAACAA
 TTAGATGCTCTTGAAAAAGAACTGCATCTAAGCTCTCAGAAATGGATTCAAT
 TAACAATCAGCTGAAGGAACTCAGAGAAAGCTATAATACACAGCAGTTAGCCC
 TTGAACAACCTTCATAAAAATCAAACGTGACAAATTGAAGGAAATCGAAAGAAA
 30 AAGATTAGAGCAAATTCAAAAAAGAACTAGAAAGATGAGGCTGCAAGGAAA
 GCAAAGCAAGGAAAAGAAACTTGTGGAGAGAAAGTATTAGAAAGGAAGAAG
 AGGAAAAGCAAAAACGACTCCAGGAAGAAAAGTCACAGGACAAAAGTCAAGA
 AGAGGAACGAAAAGCTGAGGCAAAACAAAGTGAGACAGCCAGTGCTTTGGTG
 AATTACAGAGCACTGTACCCTTTTGAAGCAAGAAACCATGATGAGATGAGTTT

TAGTTCTGGGGATATAATTTCAGGTTGATGAAAAAACTGTAGGAGAGCCTGGTT
GGCTTTATGGTAGTTTTTCAGGGAAAAGTTTGGCTGGTTCCCCTGCAACTATGTA
GAAAAAGTGCTGTCAAGTGAAAAAGCTCTGTCTCCTAAGAAGGCCTTACTTCC
TCCTACAGTGTCTCTCTCTGCTACCTCAACTTCTTCCCAGCCACCAGCATCAGT
5 GACTGATTATCACAATGTATCCTTCTCAAACCTTACTGTTAATACAACATGGCA
GCAGAAGTCAGCTTTTACCCGCACTGTGTCCCCTGGATCTGTGTCCCCCATTCA
CGGACAGGGGCAGGCTGTAGAAAACCTGAAAGCCCAGGCCCTTTGTTCCCTGG
ACGGCAAAGAAGGAGAACCACCTGAACTTCTCAAAGCACGACGTCATCACTGT
CCTGGAGCAGCAGGAAAACCTGGTGGTTTGGGGAGGTGCACGGAGGAAGAGGA
10 TGGTTCCCCAAGTCTTATGTCAAGCTCATTCTGGGAATGAAGTACAGCGAGG
AGAGCCAGAAGCTTTGTATGCAGCTGTGACTAAGAAACCTACCTCCACAGCCT
ATCCAGTTACCTCCACAGCCTATCCAGTTGGAGAAGACTACATTGCACTTTATT
CATACTCAAGTGTAGAGCCCCGGGGATTTGACTTTCACTGAAGGTGAAGAAATT
CTAGTGACCCAGAAAGATGGAGAGTGGTGGACAGGAAGTATTGGAGAGAGAA
15 CTGGAATCTTCCCGTCCAACCTACGTCAGACCAAAGGATCAAGAGAATTTTGGG
AATGCTAGCAAATCTGGAGCATCAAACAAAAAACCCGAGATCGCTCAAGTAAC
TTCAGCATATGCTGCTTCAGGGACTGAGCAGCTCAGCCTTGCGCCAGGACAGT
TAATATTAATCTTAAAGAAAAACACAAGCGGGTGGTGGCAAGGAGAGCTACA
GGCCAGAGGGAAGAAACGACAGAAGGGATGGTTTCCTGCCAGCCATGTAAAG
20 CTGCTAGGTCCAAGCAGTGAAAGAACCATGCCTACTTTTCACGCTGTATGTCA
AGTGATTGCTATGTATGACTACATGGCGAATAACGAAGATGAGCTCAATTTCT
CCAAAGGACAGCTGATTAATGTTATGAACAAAGATGACCCTGACTGGTGGCAA
GGAGAAACCAATGGTCTGACTGGTCTCTTTCCTTCAAACCTATGTTAAGATGAC
AACAGACTCAGATCCAAGTCAACAGTGGTGTGCTGACCTCCAAGCCCTGGACA
25 CAATGCAGCCTACGGAGAGGAAGCGACAGGGCTACATTACGAGCTCATTCA
GACAGAGGAGCGGTACATGGACGACCTGCAACTTTTGAACAAAAAACTCTCC
TTTGA

30

mouse Ese2 - Sequence ID NO:6

MAQFPTAMNGGPNMWAITSEERTKHDKQFDNLKPSGGYITGDQARTFFLQSGLP
 5 APVLAELWALSNDLNDGKMDQQEFSIAMKLIKLLQGQQLPVVLPPIPKQPPMFS
 PLISARFGMGSPNLSIHQPLPPVAPIATPLSSATSGTSIPPLMMPAPLVPSVSTSSL
 PNGTASLIQPLSIPYSSSTLPHASSYSLMMGGFGGASIQKAQSLIDLGSSSSTSSTAS
 LSGNSPKTGTSEWAVPQPSRLKYRQKFNSLDKMSGYLSGFQARNALLQSNLSQT
 QLATICWTLADIDGDGQLKAEFILAMHLTDMAGQPLPLTLPELVPPSFRGGK
 10 QVDSVNGTLPSYQKTQEEEPQKKLPVTFEDKRKANYERGNMELEKRRQVLMEQ
 QQREAERKAQKEKEEWEKQRELQEWEKKQLELEKREKQRELERQREEERR
 KEIERREAAKQELERQRRLEWERLRRQELLSQKTREQEDIVRLSSRKKSLHLELEA
 VNGKHQQISGRLQDVQIRKQTQKTELEVLDKQCDLEIMEIKQLQELKEYQNKLI
 YLVPEKQLLNERIKNMQLSNTPDSGISLLHKKSSEKEELCQRLKEQLDALEKETAS
 15 KLSEMDSFNNQLKELRESYNTQQLALEQLHKIKRDKLKEIERKRLEQIQKKKLED
 EAARKAKQGKENLWRESIRKEEEEKQKRLQEEKSQDKTQEEERKAEAKQSETAS
 ALVNYRALYPFEARNHDEMSFSSGDIIQVDEKTVGEPGWLYGSFQKGFGWFPCN
 YVEKVLSSSEKALSPKKALLPPTVSLSATSTSSQPPASVTDYHNVFSNLTVNTTWQ
 QKSAFTRTVSPGSVSPHGGQGAVERNKAQALCSWTAKKENHLNFSKHDVITVLE
 20 QQENWWFGEVHGGRGWFPKSYVKLIPGNEVQRGEPEALYAAVTKKPTSTAYPV
 TSTAYPVGEDYIALYSYSSVEPGDLTFTEGEEILVTQKDGEWWTGSIGERTGIFPS
 NYVRPKDQENFGNASKSGASNKKPEIAQVTSAYAASGTEQLSLAPGQLILILKKNT
 SGWWQGELQARGKKRQKGWFPASHVKLLGPSSERTMPTFHAVCQVIAMYDYM
 ANNEDELNFSKGQLINVMNKDDPDWWQGETNGLTGLFPSNYVKMTTDS DPSQQ
 25 WCADLQALDTMQPTERKRQGYIHELITTEERYMDDLQLFEQKTL

Mouse Ese2 alternative transcript partial cDNA sequence - Sequence ID NO:7

CCGTCTTCCACATTTCCACATTGATCGTGTGTACACACTCCGAACAGACAAC
 30 ATCAACGAGAGGACGGCCTGGGTCCAGAAGATCAAGGGTGCCTCAGAGCAGT
 ACATCGACACTGAGAAGAAGAAACGGGAAAAGGCTTACCAAGCCCGTTCTCA
 AAAGACTTCAGGTATTGGGCGTCTGATGGTGCATGTCATTGAAGCTACAGAAT
 TAAAAGCCTGCAAACCAAACGGGAAAAGTAATCCATACTGTGAAGTCAGCAT
 GGGCTCCCAAAGCTATACCACCAGGACCCTGCAGGACACACTAAACCCCAAGT

GGAAC TTCAACTGCCAGTTCTTCATCAAGGATCTTTACCAGGACGTTCTGTGTC
 TCACTATGTTTGACAGAGACCAGTTTTCTCCAGATGACTTCTTGGGTTCGTA CTG
 AAGTTCCAGTGGCAAAAATCCGAACAGAACAGGAAAGCAAAGGCCCCACCAC
 CCGCCGACTACTACTGCACGAAGTCCCCACTGGAGAAGTCTGGGTCCGCTTTG
 5 ACCTGCAACTTTTTGAACAAAAAACTCTCCTTTGAGGGCCTGGGGAAGCCAGA
 ACCAGGGGAGCTGCCCCACAAGGCTGGGTCTAAAGACAGATTTTGCTCTCCCAG
 GACAGAGGAGCATCACATGGCTTCATCCATCAAACAGCCACACTCGCTGGGCC
 TGTATTTTATTGCACACTAAATTGCTAGCAATCTATGCAAACATGATCTTT

- 10 Mouse Ese2 alternative partial protein containing C2 membrane-binding domains -
 Sequence ID NO:8

VFHISHIDRVYTLRTDNINERTAWVQKIKGASEQYIDTEKKKREKAYQARSQKTS
 GIGRLMVHVIEATELKACKPNGKSNPYCEVSMGSQSYTTRTLQDTLNPKNWFNC
 15 QFFIKDLYQDVLCLTMFDRDQFSPDDFLGRTEVPVAKIRTEQESKGPTTRRLLHE
 VPTGEVWVRFDLQLFEQKTL

Novel Ese-coiled-coil interacting clones:

Mouse homologue of C07E3.1 protein (clone 65): - Sequence ID NO:9

20 GAATTCGGCACGAGGGCTGAGAGAAGCGGACTCCGAGGACTCTGATGCTGAA
 GAGAAGCCTGTTAAGCAGGAGGACTTCCCGAAGATTTAGGACCAAAGAAGTT
 AAAGACGGGTGGCAATTTTAAGCCCAGCCAGAAAGGCTTTTCAGGAGGAACC
 AAGTCCTTCATGGACTTTGGCAGCTGGGAGAGACACACGAAAGGGATCGGGC
 25 AGAAGCTGCTGCAGAAGATGGGCTACGTCCCTGGGCGTGGCCTGGGGAAGAA
 CGCACAGGGGATCATCAACCCCATCGAAGCCAAACAGAGAAAAGGCAAGGGA
 GCCGTGGGGGCCTATGGCTCGGAGAGGACCACTCAGTCTCTGCAGGACTTCCC
 CGTGGCCGACTCGGAAGAGGAGGCAGAAGAGGAGTTTCAGAAGGAGCTGAGC
 CAATGGAGGAAAGACCCAGCGGGAGCAAGAAGAAGCCAAAGTACTCTTACA
 30 AGACTGTGGAGGAGCTGAAGGCCAAGGGCAGGGTCAGCAAGAAGCTCACAGC
 ACCTCAGAAGGAACTGTCTCAGGTCAAGGTGATCGACATGACAGGCCGGGAG
 CAGAAGGTGTACTACAGCTACAGCCAAATCAGCCACAAGCACAGCGTGCCCCG
 ATGAAGGGGTGCCATTGCTGGCGCAGCTGCCCCCACAGCCGGCAAGGAAGC
 CAGGATGCCGGGCTTTGCACTGCCTGAGCTGGAGCACAACTGCAGCTGCTCA

TTGAGCGCACGGAGCAGGAGATCATCCAGAGCGACCGGCAGCTCCAGTATGA
 GCGGGACATGGTGGTCAGCCTGTCGCATGAGCTGGAGAAGACGGCCGAGGTT
 CTTGCACATGAGGAGCGTGTCTCTAACCTCAGCAAGGTGCTGGCCCTGGT
 GGAGGAATGTGAGCGCCGCATGCAGCCCCATGGCACCGACCCCCTCACTCTGG
 5 ATGAGTGTGCCCCGCATCTTTGAGACACTACAGGACAAGTATTATGAGGAGTAC
 CGCCTGGCGGACCGCGCAGACCTCGCTGTGGCCATTGTCTACCCGCTCGTGAA
 GGACTACTTTAAGGATTGGCACCCCCTCGAGGG

Mouse partial C07E3.1 protein (clone 65): - Sequence ID NO:10

10

GTKSFMDFGSWERHTKGIGQKLLQKMGYVPGRGLGKNAQGIINPIEAKQRKGKG
 AVGAYGSERTTQSLQDFPVADSEEEAEEEFQKELSQWRKDPGSGKKPKYSYKT
 VEELKAKGRVSKKLTAPQKELSQVKVIDMTGREQKVYYSSYSQISHKHSVPDEGV
 PLLAQLPPTAGKEARMPGFALPELEHNLQLLIERTEQEIIQSDRQLQYERDMVVSL
 15 SHELEKTAEVLAHEERVISNLSKVLALVEECERRMQPHGTDPLTLDECARIFETLQ
 DKYYEEYRLADRADLAVAIYPLVKDYFKDWHPSR

Mouse Novel cDNA clone 42/ Est accession #W29719 and #AA915044: - Sequence ID
 NO:11

20

CATGGCGGCGGCTGCGGAGGGCGTCCCGGCGACGCGACGGAGGACGAGCCAC
 CTCGAGATGATGCTGCGGTGGAGACAGCCGAGGAAGCAAAGGAGC

Mouse Novel cDNA clone 70: - Sequence ID NO:12

25

CTTGAGTCTACTGAAAATACCCTGCAGGAAGCTACATCATCCATGTCTTTGAT
 GACCCAATTTGAACAGGAAGTATCTGGCCTCCAAAGACCATACGTGATATTGA
 GACTAGCGAAGAGATGC

30 Mouse Novel cDNA clone 83/Est accession #AA589041 and W98708: - Sequence ID
 NO:13

GAATTCGGCACGAGGGAGTCTGGTTCTGGAAAGCCGACAGAAGCTGAGCTTG
TCAACTTAGATTTCTTGGGAGATTTGGATGTTCCGGTATCTGCCCCACCCCTGT
GTGTCTGAGCTCGAGTCTCTCTGCTGGACTATGG

Novel Ese-SH3 interacting clones:

5

Mouse YNK1 partial cDNA: - Sequence ID NO:14

CTTTACGAGCAGAGGGAGCCAAATTCAGAGCCGTTTTAGATAAAGCTGTGCAA
GCGGATGGACAGGTGAAGGAGCGCTACCAAGTCCCATCGAGACACCATCGCAC
10 TTCTGTGTAAGCCGGAGCCAGAGCTGAATGCTGCCATCCCCTCTGCTAACCCA
GCAAAGACCATGCAGGGCAGCGAGGTTGTAAGTGTCTTAAAGTCCTTATTATC
AAATCTTGATGAAATCAAGAAGGAAAGAGAGAGTCTTGAGAATGACCTGAAG
TCAGTGAATTTTGACATGACAAGCAAGTTTTTGACAGCTCTGGCCCAAGATGG
CGTGATAAATGAGGAGGCTCTCTCTGTCACTGAGCTGGATCGGATCTATGGCG
15 GTCTAACAAGTAAAGTTCAAGAGTCTCTGAAGAAACAAGAGGGACTTCTAAAA
AATATACAGGTCTCACACCAAGAATTCTCCAAAATGAAGCAATCTAACAACGA
GGCTAACTTGAGAGAAGAAGTTCTGAAGAACCTAGCAACTGCGTATGACAACT
TTGTTGAGCTTGTAGCTAACTTGAAGGAGGGCACAAAGTTTTACAATGAGCTG
ACTGAGATCCTGGTCAGGTTCCAGAACAAATGCAGTGACATAGTGTGTTGCACG
20 GAAGACAGAAAGAGACGAGCTCTTGAAGGATCTGCAGCAGAGCATTGCCAGA
GAGCCCAGCGCTCCTTCAATCCCTCCTCCAGCCTATCAGTCCTCCCCAGCAGC
GGGGCATGCAGCAGCGCCTCCAACCTCCAGCCCCAAGAACCATGCCGCCTGCTA
AGCCCCAGCCTCCAGCCCGGCCTCCACCTCCTGTGCTTCCTGCAAACCGAGTT
CCTCCTGCTTCTGCTGCTGCTGCCCCCTGCAGGCGTGGGGACGGCTTCAGCAGC
25 GCCGCCACAGACCCCTGGCTCTGCTCCCCCGCCACAGGCTCAGGGACCACCAT
ACCCTACCTATCCAGGATATCCCGGGTATTGCCAAATGCCCATGCCCATGGGC
TACAACCCCTACGCATATGGCCAGTACAATATGCCGTACCCACCGGTGTATCA
CCAGAGCCCCGGACAGGCTCCATACCCAGGACCCCAGCAGCCTACCTACCCCT
TCCCTCAGCCCCCGCAGCAGTCCTACTATCCACAGCAGTAACGCTGCCACGTG
30 CTGCTGGTTCAGATCAGAGCGACAGGACAGCAGCTGCCACCAGCTCTAAGCCA
CGCTCTGGCCACTCGAGAGTATCTTGCTCTATTGATTGCTGTGGATGATTTCTG
TCTGTGGCTAAAGCCGAAGGCTGGGCCCCACCTCCACATTTGATCGCACTCGT
GAGATTCTGCTGCTGTTGCAGTATAAACGCTAGCTATAATAGCATTTGAAAAA
AATTACAGTTCCATAAAATGCTGAAAATGAGAAATTAAACCTGCAAGTGAAAC

ATTTGAAATTAGCATACTTTATAAGATGCAGTTGGGACAAAGATGGCTTAAGT
 ACTGATATTTAAGGAAAAAGTTTTCTTTCTCTTTTGGTTTATTGATTTAGTTTA
 ATTTCTATTATGATATTTTGCATAATCAAGGCATTGTAAATCTTATAATTTAAA
 AATAAATTACTTACGAACAGTTGTCATTGTTATGTTTTGTCATTGATTCTCATT
 5 GCTGTCTAGTTCCTTTCTGGTATTAGCCTCTCCTTCTGTATGTTACAGGCTCC
 ATTACTGTGTTGAATTGCGTGACGTCAGGTGAGCAGTCAGGGAGGGCTGCTCT
 GCGGACGCCAAGCGCACACCAGCTTGTCTCAGGCTCAGCAGTCAGCTCATCTG
 GACATTTCTATTTAAAAAGTCCTTTAATGTGGAAGATACACACAATTGTTACCA
 AAGGTTCTTCCAATTAATTTTACAATTTAAAAAGTATGTATTAATGTTTTATTG
 10 TTAGATTTTCCAAAAAATGATGCAAATTCTGGTAATATTCATTTCCCTCACCC
 ATAATTTGGTTAAAATGAGTAGTTTTAGCCATACAGTCTCATCTGCTGTGGAG
 GAACCTGGAGAAAGTCCCCTGTGCCTTTCTAGCCCTTGGGTTCTATTCTTATCC
 TGCAATGTCTACTGCACAGTGTGTTTGAGCAGATCCTAACCCTCCTTTTACAGT
 TTCTTCTTCTTACTTCTTTATTCTTTTTGTGGCTCCTGAAATCTGAGGTTATTTT
 15 GTAATTCAGGAGCATGCAGGACAATTGTTGGGACATGTGCCTAGTCCGGAATA
 CAGCCCAGGACAGCAAGGAGATGCGTCCTGCACCAGGAAGCCGTGCAGGCAG
 GAGCTGTCCAAGGTCCCGGCGGCTCTGCCTGTGTGAGGCAGGAGAATGAGCA
 GATTCCCTAATCTATGTTCTCGAAGTTTAATGCTGATGTTGTCTTGCCTTATCC
 TCATTTAACTGATACTGTCAACCAGTCCACCTTTGCTCTCATTGCAAAGTGATA
 20 GTGTAATTTCAAATGTAAGACTGAAGATACGATTGTAAAAGGGAGTAACTGG
 TTAAACGTGTTATTCTAAAGCACCTTACTTTGTTGTTGTATGCAGAAAACACA
 GATGCGCTAATTCAGTATAAATGACTGATTGCCTGGAATTTGGACGTTGGCTT
 AAAGTCCGATAGCTAAACCTTGGCAAAACATAACAAACATTTTCATTGCTCAGC
 CTCAGTGCTCTGGAGTATTCAGTGTATGAGACAGGTTTATTTGAGTCCTCTGTA
 25 AATGGCATTGTAATTTTATATTCTCCCCTCCCGAGTATCTTATAAGACATCCCC
 TGAGTTAGGGAGTTCCAGACTGCTACTCTATTCCTTATGAATGCAAAACAAC
 CACCAATAGAACAAAAAAAAAAAAAAAAAACTCGAG

Mouse YNK1 partial coding cDNA - Sequence ID NO:15

30 CTTTACGAGCAGAGGGAGCCAAATTCAGAGCCGTTTTAGATAAAGCTGTGCAA
 GCGGATGGACAGGTGAAGGAGCGCTACCAGTCCCATCGAGACACCATCGCAC
 TTCTGTGTAAGCCGGAGCCAGAGCTGAATGCTGCCATCCCCTCTGCTAACCCA
 GCAAAGACCATGCAGGGCAGCGAGGTTGTAAGTGTCTTAAAGTCCTTATTATC

AAATCTTGATGAAATCAAGAAGGAAAGAGAGAGTCTTGAGAATGACCTGAAG
 TCAGTGAATTTTGACATGACAAGCAAGTTTTTGACAGCTCTGGCCCAAGATGG
 CGTGATAAATGAGGAGGCTCTCTCTGTCACTGAGCTGGATCGGATCTATGGCG
 GTCTAACAAGTAAAGTTCAAGAGTCTCTGAAGAAACAAGAGGGGACTTCTAAAA
 5 AATATACAGGTCTCACACCAAGAATTCTCCAAAATGAAGCAATCTAACAACGA
 GGCTAACTTGAGAGAAGAAGTTCTGAAGAACCTAGCAACTGCGTATGACAAC
 TTGTTGAGCTTGTAGCTAACTTGAAGGAGGGCACAAAGTTTTACAATGAGCTG
 ACTGAGATCCTGGTCAGGTTCCAGAACAATGCAGTGACATAGTGTTCACG
 GAAGACAGAAAGAGACGAGCTCTTGAAGGATCTGCAGCAGAGCATTGCCAGA
 10 GAGCCAGCGCTCCTTCAATCCCTCCTCCAGCCTATCAGTCCTCCCCAGCAGC
 GGGGCATGCAGCAGCGCCTCCAACTCCAGCCCCAAGAACCATGCCGCCTGCTA
 AGCCCCAGCCTCCAGCCCGGCTCCACCTCCTGTGCTTCCTGCAAACCGAGTT
 CCTCCTGCTTCTGCTGCTGCTGCCCCTGCAGGCGTGGGGACGGCTTCAGCAGC
 GCCGCCACAGACCCCTGGCTCTGCTCCCCCGCCACAGGCTCAGGGACCACCAT
 15 ACCCTACCTATCCAGGATATCCCGGGTATTGCCAAATGCCCATGCCCATGGGC
 TACAACCCCTACGCATATGGCCAGTACAATATGCCGTACCCACCGGTGTATCA
 CCAGAGCCCCGGACAGGCTCCATACCCAGGACCCCAGCAGCCTACCTACCCCT
 TCCCTCAGCCCCCGCAGCAGTCCTACTATCCACAGCAGTAA

20 Mouse YNK1 partial protein - Sequence ID NO:16

LRAEGAKFRAVLDAVQADGQVKERYQSHRDTIALLLCKPEPELNAAIPSANPAKT
 MQGSEVVSVLKSLLSNLDEIKKERESLENDLKSVMFDMTSKFLTALAQDGVINEE
 ALSVTELDRIYGGTSTKVQESLKKQEGLLKNIQVSHQEFKMKQSNNEANLREEV
 25 LKNLATAYDNFVELVANLKEGTFYNELTEILVRFQNKCSDIVFARKTERDELLK
 DLQQSIAREPSAPSIPPPAYQSSPAAGHAAAPPTPAPRTMPPAKPQPPARPPPPVLP
 ANRVPPASAAAAPAGVGTASAAPPQTPGSAPPPQAQGPPYPTYPGYPGYCQMPM
 PMGYNPYAYGQYNMPYPPVYHQSPGQAPYGPQPQPTYFPQPQQSYYPQQ

30 Mouse novel cDNA clone 4: - Sequence ID NO:17

GGTCTTGGCTAGAATTTTAAATTTCTTCTCATTTGAGTAAAATGTTGCATTCTG
 AAGTCCCATGCTACCTGAAGTTGCATTTGGAGTCCCAAGCTACTGGAATGTTT
 ATATGTGACCGTTTCCAGGAGGCTTACACTGCAGAAGGAAGAATGAATCTAG

GTGAGGTGGGCAGCTGCTTGGCAGTCCTCTCTTGTGCCCCAACTGTAAACCAG
ATAGAAATGTTTCAGGGGAGGATACTTTTCATTATTGTGGTTTGTAGTGTTAAGA
TGATTGCTTCTGCCTTGGAAATACCTCAAGCTGTTCTTATTTAACAGGTAAGTG
ACTGAGTATAATATTCCAGAAAAATTTGAAATCCTAATTTCTTCCATATTTTCAT
5 TAAATTTTTTGCATACAGGTCTAACAAATATGGATATGTATACACATCCTCTTT
AATGAAGGTATTATTTTGGTTACTTTTCCTAAGATATACCTTAAAAGATGTTCT
ATACATTTCTACTTAAATTCTGGGGGATTGGAGTATGTACATGATAAAAAA
GATTATAATATATCGATTGAAGTTACTTTATTTTCTAATTAGAATTATTTTAAT
AGTCCTTTATTGAATAAGTGCTGTAATTTGTTTGCTATGAGACTTATTCCTGAT
10 GTGAATGTAAATTATTTTCCACATGCATGAAAAAATGTATGTACTAATCAGA
GTTGTCTCCATTGCATTGAAATTACTTGTTTTGAAGTAAAGTAACTCATATTTA
TGTAGTAGAATGCTTATGTTTTCAGACTTTGTAATGATTTCTTTGGATGTATT
TTAAATCAATCGGTCTGGGTAACATATCAGTTTAGATTAATATGTGCTTAAAA
GAAGAAAAAAATTCAATGGTTCATAGTAGAAATGTGCCACACTTAAATAAGCT
15 CTGTATGACATGAAATTCTGTAAAACATTGTAATTCATGGTGACTTTTAACTT
ATAAAAAATACTACTTGCACGGGTTACTTGATTTATGGATATATGAAAACCTTCTC
AGGACGAAAGTTCTTCTTTCTCTAGAACTATTCTTCTGTCGGTCATGCAGAATG
CTGTTATTCTGAAAAGTGTCCTGTTGCATATGATGGTCACTTTATTTGGGGGG
ATTCTTCATAAGATGTGAGATGTTGATGCCAGTCTTTCCCAAGTAAGTGCTCGT
20 AAAAAAGGACTACTAACTAGCCTGCATCTGTCTCTAACTGGGACCAAGGGGTC
TGCTGAAGGAAACTGAAGAGCTCTAACATTTTCACAGCTTGGAGAAGATAGAA
TCTTTAAAAGTACAACTGAAGCTTGATCTATTTTACAAGTGCATTGATGGCCCC
TGTCCTTCTCTGGTTCCTGTCATTTGAAACCAACTCCTGTTGTAAATAGGAAGA
ATATGGGACATTCATATTTAAGAAAATTTGATGTCATTAGGTGACTAAGTAGA
25 AGGCTTAGAAAAATGTATTCATTTGCAAGTATTTTGGCACAAGAAATTTTCCA
ACTGAATAGTAAGCAAAAGCTAAGTTGTTTCATTGAAATCATAAGGCAGTTTA
AGATAAACTGGAGAAGATAACTGTTCTAATAGAGGATAATCGAATTGATTGTC
AAGTGGATGTTATTTATTGGATAGTGACAGAGTTTATTTGTAACCTTAATTATA
TTAAAAGTTATTCTGTTAGGATGTTTTGTATTAATAAACGTGAACAAAATTAA
30 AAAAAAAAAAAAAAAAAAACTCGAGGG

Mouse Novel cDNA clone 8/47/52: - Sequence ID NO:18

5 GAGAAGGCGGCCTGCCGCAGCGGGACAACCTAGAGCGCGACGTGGAGGCGCG
 TAGCGGAGCTGGAGCAACTGCGCACCGAGGTGGATGTGCGCATTAGCGCNNT
 GGACACCTGCGTCAAGGCCAAGTCGCTGCCAGCCGTCCCGCCGAGAGTCTCAG
 GCCCACCCCCGAACCCTCCACCCATTGATCCAGCTAGCCTGGAGGAATTCAAG
 AAAAGGATCCTGGAGTCTCAGCGGCTCCCTGTAGTCAACCCTGCWGCCCAACC
 CAGCGGTTGAGRACCCAGCTGCCGCAGGACGCTGGGTGCCAGAATCGCCAC
 10 CTGTGGATGGGGGCAGCCAGGTGCCACAGTGCTGGACACCCGCCGTGCCTG
 CCGGCAGCCTCCACCCCCAGCGCCTTCTCTGGCACCCCTTCACTGTCCCSTGCA
 TCCCCRCCATTCCSSCASWSASKGGATTAAAGGCACACACAGCTGTGAGATGACT
 TCACATCGACCCCTTGTGCAGTGACCCGGATGGTGCCCCACCCACACATGAAG
 CACCCACAGCTCAGCTGCCACCCTAGGCAACTCCTCCGGTTTCCTATCACTCTG
 15 CTCCTGACCCGGGAGGTGAGAACAGGAAGCCCAGCCTTCAGCTCCCTTGGGAG
 TTTCCAGCCTCCCTCTTAAAGGCCACTAGGGTTTCCAGATCCTATTTGAGAGTC
 TCCAGGCCTCCCCTGAAGGGTTCTAGCCACCACGCCCACAGGATTCCCATTAG
 GTTTTAAAGTCTTTTCCAGAGTCCGCTGGTTCCCCTCCTCCTCACAAGGAAGGG
 CCTCAATTGTAGATGAGCGTTCCGGGTGGATCTTAGAGCCCTAGAGGGAGGCT
 20 TTTGCTTG TARCCCCCTAAAGATATTACTGGCACATAATAAATATGAAAGTCCT
 TTGAAA

 GTTGGACACTGCGCAAATGGGGCTCTCCATGGACCGCAGCCATACGCCCCGA
 CGGGGGACCAGCAGCGCGGCTCTGGTTCTACCTGCGCTATTTCTTCCTCTTCGT
 25 GTCGCTCATTAGTTCCTCATCATCCTGGGCCTGGTCCTCTTCATGATCTATGG
 CAATGTGCACGCCACCACTGAGTCCAGCCTGCGCGCCACGGAGATCCGCGCCG
 ACAGCCTGTACAGCCAGGTGGTTGGACTATCGGCCTCACAGGCTAACCTGAGC
 AAACAGCTGAACATCAGCTTGCTTGTC AAGGAAACAGTCATGCAGCAACTGTT
 GACTACGCGACGTGAGATGGAGCGCATCAACGCCAGCTTCCGCCAGTGCCAA
 30 GGCGACCTGATCACCTACATAAACTATAATCGCTTCATCGCCGCTATCATCCTG
 AGCGAGAAGCAGTGCCAGGAACAGCTGAAGGAGGTCAACAAGACCTGCGAAC
 TTTACTCTTCAAGCTGGGAGAAAAAGTTAAGACACTGGAGATGGAGGTGGCC
 AAGGAGAAGGCAGTGTGCTCCAAGGACAAGGAGAGCCTGCTGGCAGGAAAGC
 GGCAGACGGAAGAGCAGCTGGAGGCCTGTG

Mouse Novel cDNA clone 18/25 - Sequence ID NO:19

5'end of partial clone

5 TGTGCGCCGCCTCTAGAACTAGTGGATCCCCCGGGCCTGCAGGAATTCCGGCA
 CGACGGCCGAGCGCCGCGGACCAACCCGCGGCTGCCCGCCGAGCCGTCGACAT
 GTGGGGGGGACTGGGGTGGGAGCGGCCGGAGCAGCGCCAGGTACCCGGGCGC
 GCAGAACCATGGCTCTCGCTCGCCTGTCCTGACCTGGCTTGCTCGCCCCACCG
 AAGAATGTCAGCCAAGTCCAAGGGGAACCCTCCTCGTCCTCCGCAGCCGAGGG
 10 ACCGCCGGCAGCCTCCAAAACCAAGGTGAAGGAGCAGATCAAGATCATAGTG
 GAGGATCTGGAATTAGTCCTGGGCGACCTGAAGGACGTGGCCAAAGAACTTA
 AGGAGGTGGTTGACCAGATTGACACCCTGACCTCTGATCTACAGCTGGAAGAT
 GAGATGACCGACAGCTCCAAAACAGACACTCTGAACAGCAGCTCCAGTGGGA
 CAACAGCCTCCAGCATAGAGAAGATCAAAGAACAGGCCAATGCTCCCCCTCATT
 15 AAACCTCCAGCACACCCGTCTGCTATCCTGACTGTCCTGAGAAAGCCAAACCC
 TCCACCGCCTCCTCCAAGGTTGACACCCGTGAGGTGTGAAGAGCCTCAGAGAG
 TGGTGCCGACTGCCAACCCCTGTAAAGACCAATGGCACTCTTCTGCGGAATGGA
 GGCTTAGCGGGGAGGCCCAACAAAATTCCAAATGGAG

20 3' end - Sequence ID NO:20

CTCGAGTTTTTTTTTTTTTTTTTTTTTTTTTTTCATTATTTACTATTATTTATTGA
 CATATTTCCAAAGCTCAAAATATTTTATTATACATATAGTTGAACATATGTTTC
 AAATTGTATAGTATAGAAAATAAACTTTTTTGTAGTGTCTCAGCATTTCATGA
 TGCAAAACTATTGACAAACATCTTTAGAAAAATAATAAAATAGTCCTTCGGTA
 25 TTAAAATTCTTATTA AAAAGCATTAGATCAAAGGGAGAACTATGACATCATCA
 ATGCATAGATGAGATAGGCATGAATGGAATGAGTTGCCCTGGCTTTATCAACA
 AATCAAAATATCTGACATCCCAGCTCTTATAATAGACCAAAATACTTGGAATC
 AGAAGGTCACAGTTTGTTTTAGGTCAATCACAAAAAATAAAATTCATTTCATA
 CTTTCTCAATTTTCCGCAGTTTCTGATGATGGAACATAGAAAACAATGTACGTC
 30 CAGGACAGAGGCGCTACTCTGCATACTTACCACGTGATTTTTTATGCCACTTTG
 TTGAATGCAGATTAATATATTTGGGCTTTTTATTGCTTGAGTAGAAAGTGCTCA
 TTA CTTATTATTTTACGTTTATCATATAGAAAATTAAAAACAAACAGAACGTTT
 TCTTAAATGGCAGATATCACACTGTGGTAGTGGTGGATTTCCTCAGGATGGTC

TTCTGTGGTTTTGGTGCAGCGGGAGGAGGCACGGTTGCAGGTGTGGGAGGGG
GGAAACTGTTACTGTGGCTTATTCCCAGTCCCCCATTTTCTAATGGGAAAT

Mouse Novel cDNA clone 95/ EST accession #AA119951 - Sequence ID NO:21

5

GCACAGCCCCCTCCATCCTGAAGAAAACCTCAGCGTATGGGCCTCCAGCTTC
GGGCCGTGTCTATCCTTCCTCTCCTGGGACATGGTGTTCCCCGCTTGCCCCCCT
GGCAGAAAACCG

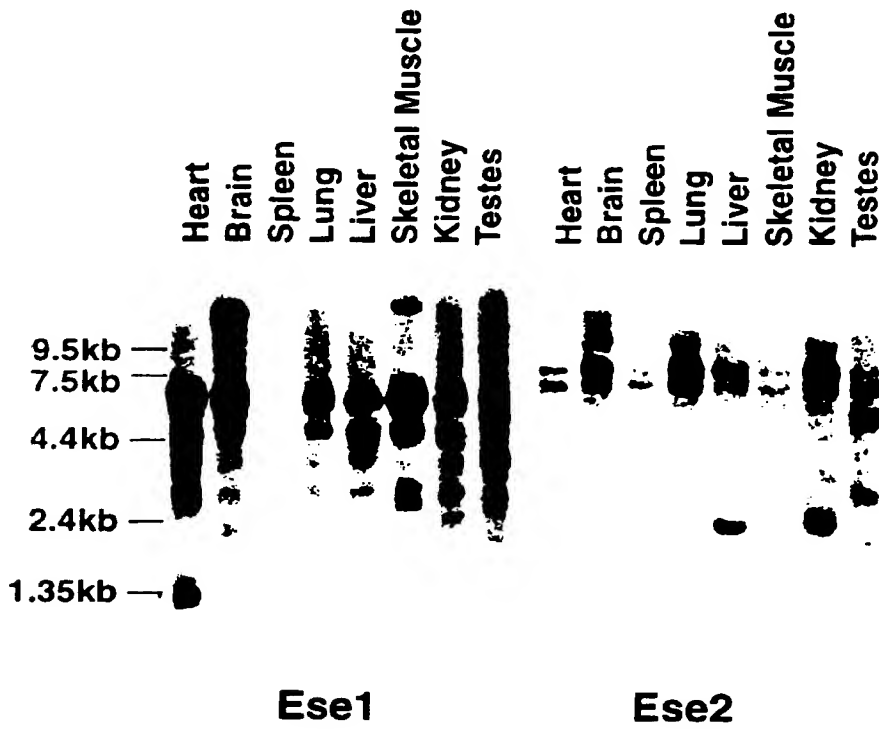
Ese1 MAQFPTPFGGSLDVWAITVEERAKHDCOFLSLKPIAGFITGDOARNFFEC
:::
Ese2 MAQFPTAMNGGNMMAITSEERTKHDKOFDNLKPSGGYITGDARTFFLO

```
C-C      TLEFELEALNDKKHQLEGLQDIRCLATQRQEIESTNKSRELRIAEITH
          :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :
          SLHLELEAVNGKHQQISGRQLQDVQIRKQTQKTELEVLQKQCDLEIMEIKQ

          LQQQLQESQOMLGRLIPEKQILSDQLKQVQQN--SLHRDSLTLTKRALEA      573
          :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :
          LQQELKEYQNKLIYLVPEKQLLNERIKNMQLSNTPDSGISLLHKKSS-E-      587

          KELARQQLREQLDEVERETRSLQEIQVFNQKLKELREIHSKQQLQKQRS
          :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :  :
          KEELCQRLKEQLDALEKETASKLSEMDSFNNQKLKELRESYNTQQLALEQL
```


FIGURE 1



Anti-Ese1 Immunoprecipitations

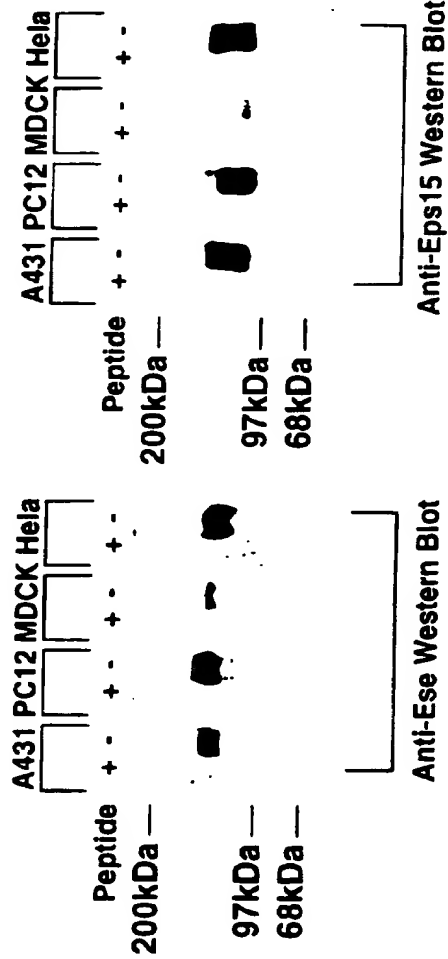


FIGURE 2

FIGURE 3

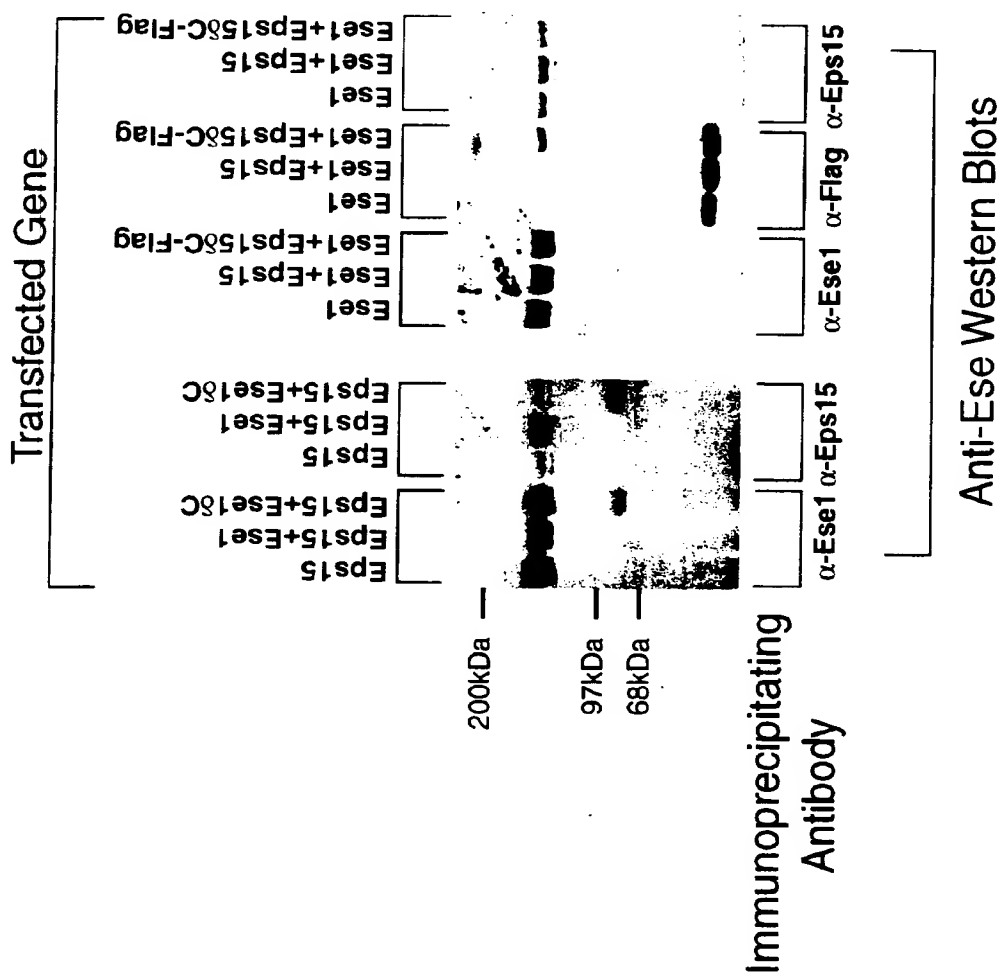


FIGURE 4

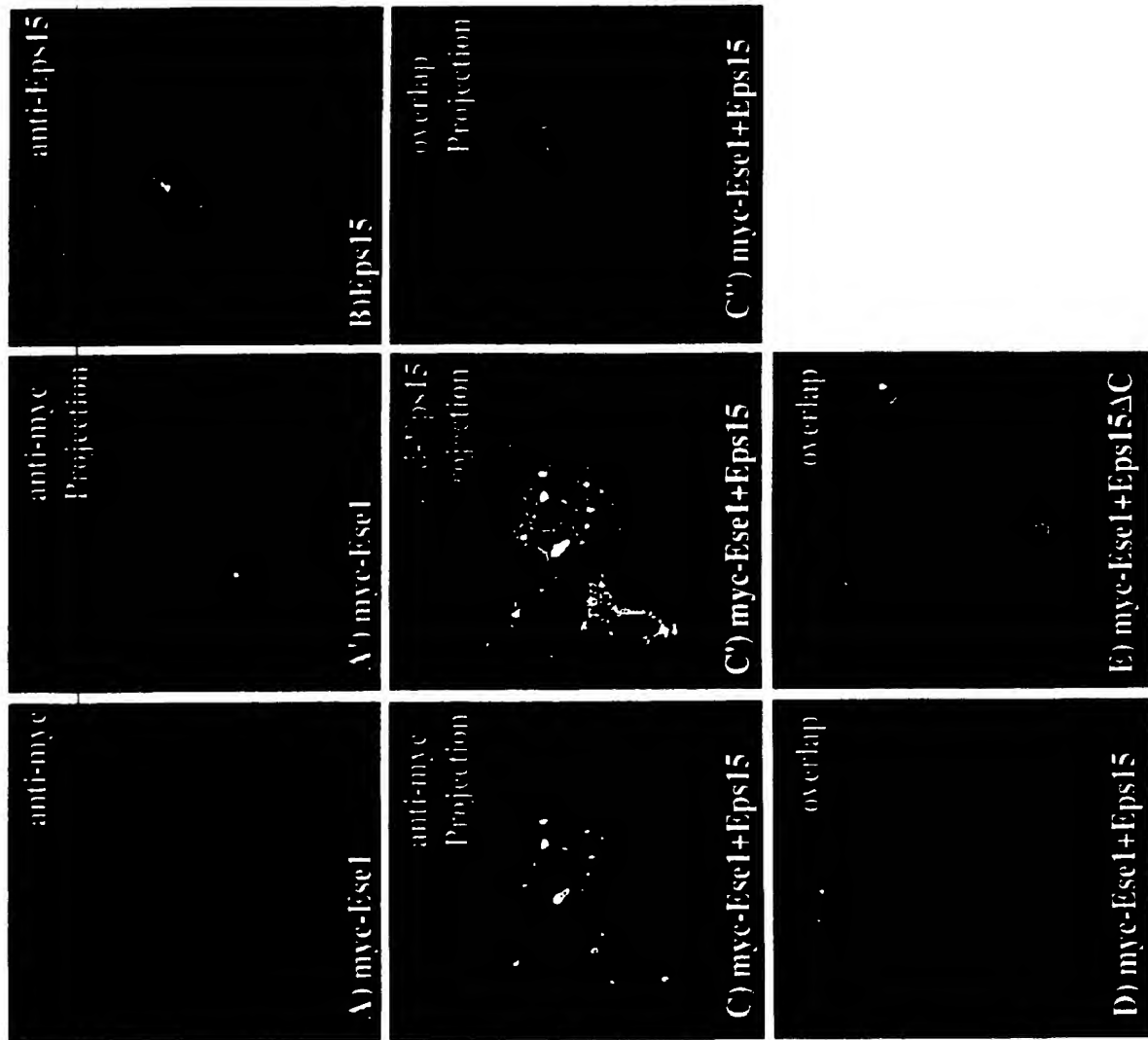
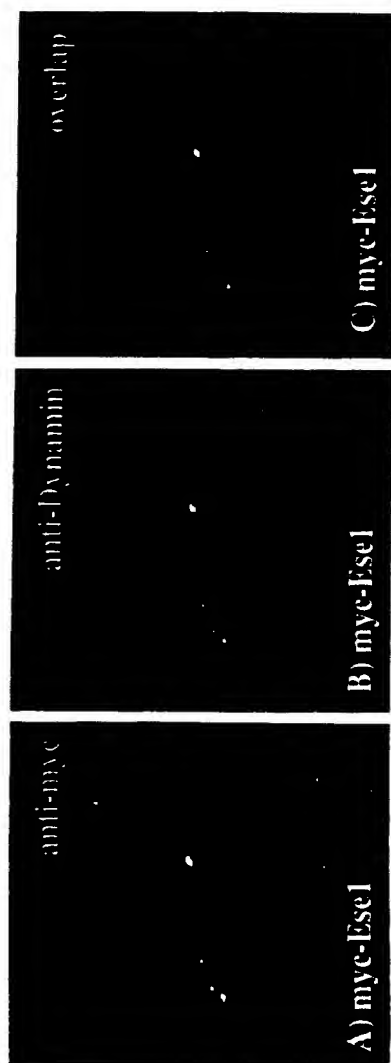


FIGURE 5

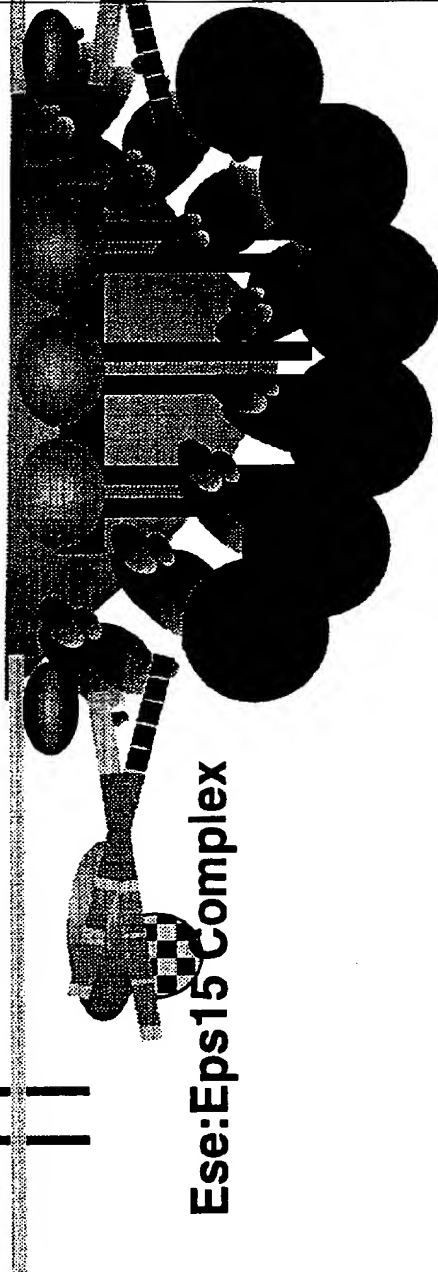


Model: The Ese:Eps15 complex recruits critical components for coated pit formation and scission

Receptors



Ese:Eps15 Complex



Activated Receptors

Clathrin coat assembly



FIGURE 6

